From DEPARTMENT OF ONCOLOGY-PATHOLOGY
Karolinska Institutet, Stockholm, Sweden

REGULATORY MECHANISMS CONTRIBUTING TO THE HOMEOSTASIS OF NORMAL AND MALIGNANT HEMATOPOIETIC CELLS

Harsha Madapura Sekharappa

Stockholm 2016

Published by Karolinska Institutet.
Printed by Eprint AB 2016

ISBN 978-91-7676-300-1

Regulatory mechanisms contributing to the homeostasis of normal and malignant hematopoietic cells

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Harsha Madapura Sekharappa

Principal Supervisor:
Professor Klas G. Wiman
Karolinska Institutet
Department of Oncology-Pathology

Co-supervisor(s):
Professor Emeritus Eva Klein
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Noemi Nagy
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Daniel Salamon
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Opponent:
Professor Eva Rajnavolgyi
University of Debrecen
Department of Immunology

Examination Board:
Professor Birgitta Sander
Karolinska Institutet
Department of Laboratory Medicine
Division of Pathology

Professor Gunilla Enblad
Uppsala University
Department of Immunology, Genetics and Pathology; Experimental and Clinical Oncology

Associate Professor Katja Pokrovskaja Tamm
Karolinska Institutet
Department of Oncology-Pathology

To my family

ABSTRACT

Bone marrow is the site of origin of diverse population of hematological cells with lineage specific function. The different lineages of cells originate from a common progenitor that has a strong self-renewable capability giving rise to myeloid and lymphoid progenitor cells. The myeloid progenitor cells terminally differentiate to monocytes, granulocytes, erythrocytes and thrombocytes, while the lymphoid progenitor cells terminally differentiate to B, T and NK cells. A plethora of extracellular and intracellular factors in a complex yet tightly regulated orchestra controls the genesis and function of each cell type. However, breakdown of one or more regulatory processes can steer cells towards becoming cancerous, characterized by the loss of normal cellular functions and/or morphology. This thesis analyses; The contribution of p53 in the regulation of normal T cell homeostasis; A mechanism evolved by EBV for its sustenance in B cells and, a mechanism contributing to the development/survival of malignant hematopoietic cells.

The first half of the thesis describes the contribution of the tumor suppressor p53 in the regulation of T cell homeostasis. Paper I demonstrates the regulation of pro-apoptotic SAP by p53 in activated T cells. p53 and SAP were upregulated in activated but not in unstimulated T cells. To determine the effect of p53 on SAP expression, p53 was selectively induced by nutlin-3 in unstimulated T cells. This induced SAP mRNA and protein expression. Further, chromatin immuno-precipitation confirmed the binding of p53 to the SAP promoter in activated T cells. Paper II demonstrated the cMyc-p53 feedback mechanism contributing to T cell homeostasis. cMyc, p53 and p14ARF expression was induced in activated T cells. Further elevation of p53 in activated T cells by nutlin-3 treatment decreased cell proliferation, cMyc and p14ARF expression. At the other end of the feedback loop, inhibition of Notch (DAPT) signaling or cMyc (10058-F4) in activated primary T cells decreased proliferation accompanied by decreased cMyc, p14ARF and p53 expression. Thus, inhibition of cMyc expression had a negative outcome on p53 expression, and induction of p53 had a negative consequence on cMyc expression in activated T cells. Nutlin-3 induced p53 and 10058-F4 mediated cMyc inhibition in activated T cells had a combined effect of apoptosis and cell cycle arrest. On the contrary, activated T cells treated with nutlin-3 or cMyc inhibitor (10058-F4) retained the cytotoxic function despite the diminished proliferation.

The second half of the thesis explores mechanisms that virus infected and tumor cells exploit for their manifestation. In paper III we studied the role of CD4+T cells in the establishment and regulation of EBV latency. Our results showed that activated CD4+T cells from healthy donors downregulated the latency III protein EBNA2 (and its transcript, Cp) in LCLs, thus indicating that they induce a shift in latency towards II. By exploiting the transwell system we demonstrated that cell-cell contact is not essential to induce the latency shift, and that soluble factors produced by activated CD4+ T cells contributed to this effect. We also identified two soluble factors, IL21 and soluble CD40L that facilitates the latency shift.

In paper IV we analyzed the effects of IFNγ on BCL6 expression in chronic myeloid leukemia and multiple myeloma cells. IFNγ or imatinib treatment alone marginally induced BCL6 expression in CML cell lines and primary CML stem cells. On the contrary, combined treatment with IFNγ and imatinib induced remarkable BCL6 induction in a direct, STAT1 dependent manner. We also demonstrated that the sustained STAT5 activation is responsible for the low BCL6 expression in IFNγ only treated CML cells. IFNγ treatment alone in MM cells strongly induced BCL6 expression through STAT1 signaling. Interestingly, IFNα only marginally upregulated BCL6 in MM cells in spite of the strong STAT1 and STAT3 activation. We show that this effect is consequence of the concomitant IFNα induced phosphorylation of STAT5. Our finding regarding BCL6 upregulation, elucidates the adverse effect of IFNγ on the outcome of tyrosine kinase inhibitor therapy in CML. Furthermore, this may partially explain the inefficient therapeutic effects of IFNγ in MM.

LIST OF SCIENTIFIC PAPERS

I. **Madapura HS**, Salamon D, Wiman KG, Lain S, Klein G, Klein E, Nagy N. p53 contributes to T cell homeostasis through the induction of pro-apoptotic SAP.
Cell Cycle. 2012 Dec 15; 11(24):4563-9.

II. **Madapura HS**, Salamon D, Wiman KG, Lain S, Klein E, Nagy N. cMyc-p53 feedback mechanism regulates the dynamics of T lymphocytes in the immune response.
Cell Cycle. 2016 Mar 17:0. [Epub ahead of print]

III. Nagy N, Adori M, Rasul A, Heuts F, Salamon D, Ujvari D, **Madapura HS**, Leveau B, Klein G, Klein E. Soluble factors produced by activated CD4+T cells modulate EBV latency.
Proc Natl Acad Sci USA. 2012, 109:1512-7.

IV. **Madapura HS**, Noemi Nagy, Dorina Ujvari, Tomek Kallas, Marijke Kröhnke, Sylvie Amu, Magnus Björkholm, Leif Stenke, Pijus K Mandal, John S McMurray, George Klein, Eva Klein and Daniel Salamon. Interferon γ is a strong, STAT1 dependent direct inducer of BCL6 expression in multiple myeloma and in imatinib treated chronic myeloid leukemia cells. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

ABL	Abelson murine leukemia viral oncogene
ACAD	Activated T cell autonomous death
AICD	Activation induced cell death
BART	BamH1-A rightward transcript
BCR	B cell receptor
BCRBL	Burkitt lymphoma
CDKs	Cyclin Dependent Kinase
ChIP	Chromatin immune-precipitation
CLL	Chronic lymphocytic lymphoma
CML	Chronic myeloid leukemia
CSR	Class switch recombination
CTL	Cytotoxic T lymphocyte
DISC	Death inducing signaling complex
DNA	Deoxyribose nucleic acid
EBNA	EBV Nuclear Antigen
EBV	Epstein-Barr virus
FADD	Fas associated protein with death domain
c-FLIP	Cellular FLICE-inhibitory protein
c-FLIPL	Cellular FLICE-inhibitory protein long
c-FLIPS	Cellular FLICE-inhibitory protein short
HPV	Human papilloma virus
IAP	Inhibitors of apoptosis proteins
DLBCL	Diffused large B cell lymphoma
GC	Germinal center
HLA	Human leucocyte antigen
HSC	Hematopoietic stem cell
IFN	Interferon
IL	Interleukin

IM	Infectious mononucleosis/Imatinib mesylate
IRF	Interferon regulatory factor
JAK	Janus kinase
LMP	Latent membrane protein
LCL	Lymphoblastoid cell line
MAPK	Mitogen activated protein kinase
MM	Multiple myeloma
NK	Natural killer
TNF	Tumor necrosis factor
PTLD	Post-transplant lymphoproliferative disease
RNA	Ribose nucleic acid
SAP	SLAM associated protein
SH2	Src homology
STAT	Signal transducer and activator of transcription
Treg	Regulatory T cells
XLP	X-linked lymphoproliferative disease

1 INTRODUCTION

1.1 Cancer

Cancer is a generic terminology for a large group of diseases manifested by abnormal division or proliferation of cells. The diseased cells - tumor cells can originate from almost all tissue types in humans and are different from the normal cells in one or more ways like, function and appearance. Genetic alteration is the fundamental requirement but most often does not by itself initiate tumor. Exposure to harmful radiations, genotoxic substances and viral infections are a few examples that can induce genetic alterations such as mutations, deletions or amplifications. Normally, cells with genetic alterations or cells infected by virus are eliminated by 'self-induced' cell death – apoptosis, or killed by immune cells following its recognition - immune surveillance [1], but tumor cells are capable of bypassing such regulations. Hallmark capabilities of tumor cells to bypass such processes are summarized by Hanahan and Weinberg as follows [2]. (i) Uncontrolled and sustained cell proliferation. (ii) Evasion of extra cellular signals that suppress cellular growth. (iii) Avoiding recognition and thus elimination by immune cells. (iv) Resistance to programed cell death. (v) Potential for unlimited replication, leading to immortality. (vi) Ability to procure nutrition by stimulating the formation of new blood vessels. (vii) Alter cellular metabolism to suite high energy and cellular substrate requirement. (viii) Loss of anchorage and increased invasiveness that helps cell metastasis. (ix) Increased genomic instability and accumulation of mutations. (x) Ability to induce inflammatory responses that helps cell proliferation by providing the necessary growth signals.

Genetic alterations in broadly two groups of genes contribute to the pathogenesis of tumors: (i) Proto-oncogenes: Those that manifest tumor development and progression. Example: The translocation of proto-oncogene cMyc, renders it the oncogenic property and as a consequence drives B cell proliferation in Burkitt lymphoma [3] (ii) Tumor suppressor genes: Example: p53, the most studied gene is also the most common gene mutated in about half of all cancer types [4]. Mutation or deletion of p53 abrogates its ability to induce cell cycle arrest, induce cell death by apoptosis and other less understood mechanisms that are essential in response to oncogenic stress or DNA damage.

According to a recent report by World Health Organization (WHO), cancer causes an estimated 13% of the deaths worldwide, with a 70% increase in new cancer cases expected in the next twenty years, making it a high priority to find long lasting cure for the disease.

1.2 Tumor suppressor p53

p53 was discovered by David Lane and Arnold Levine while studying tumor inducing SV40 viral oncoproteins in mouse cells where it was recognized as a 53 and 54 kDa protein respectively in complex with the SV40 large T-antigen [5, 6]. The tumor suppressor role of p53 was realized much later in studies comparing the effect of expressing wild type and mutant p53 cDNA in transformed rat embryo fibroblasts [7]. The loss of p53 function, either

by gene mutations/deletion or by the physical interaction of viral proteins such as, human papilloma virus (HPV) protein E6 [8], Human T-lymphotropic virus (HTLV) Type I – Tax protein [9] Epstein-Barr virus (EBV) – EBNA-5, -6 and BZLF1 [10-12], renders the cell more vulnerable to oncogenic stress induced tumor development. Studies on whole genome sequencing confirm that p53 is the most commonly mutated gene spanning all cancer types, with a 42% mutation frequency across 12 common types of tumors and above 90% frequency in certain high grade tumors [4]. p53 is activated in response to DNA damage by oncogene activation and aberrant DNA replication which leads to a selection pressure for p53 mutations during tumor development [13, 14] . Numerous findings imply that sustained p53 function is essential to regulate cell proliferation and for the prevention of cancer.

1.2.1 Regulation of p53

p53 plays a critical role in regulating cell cycle progression and apoptosis. However, undesired elevated p53 levels can inhibit normal cellular growth, making it important to regulate its expression. In most cell types p53 expression is negatively regulated at the protein level by proteasomal degradation. MDM2 is the principal E3-ligase that binds to p53, ubiqitinating it for proteasomal degradation [15]. Six lysine residues at the carboxyl terminal of p53 are the predominant sites for MDM2 facilitated ubiquitinalation [16]. Apart from MDM2, other ubiquitin ligase such as Pirh2, COP1, Arf-BP1 and RNF3 [17-19] have also been shown to promote p53 degradation trough ubiqitination.

p53 is induced as response to various types of cellular stress like oncogenic stress, DNA damage, hypoxia, nutrient depravation, telomere attrition and high levels of reactive oxygen or nitric oxide. Posttranslational modification of p53 is one of the mechanisms to elevate p53 level. Phosphorylation of p53 is often described as the crucial step towards p53 protein stabilization [20]. Eleven serine residues in the transactivation domain at the amino terminal of p53 are shown to be important sites for phosphorylation leading to the dissociation of the MDM2-p53 complex, thus stabilizing p53 protein. In response to cellular stress signals, a broad range of kinases such as, ATM, ATR and Chk1/2 are responsible for the phosphorylation of multiple serine residues on p53 [21-24]. In addition to phosphorylation, p53 can also be acetylated resulting in the destabilization of the MDM2-p53 complex [25]. Acetylation of p53 is brought about predominately by CBP/p300 acetyltransferases on the carboxyl terminal lysine residues of p53 [26].

p53 is also subjected to additional post translational modifications such as, methylation [27], sumoylation [28] and neddylation. These modifications of p53 contribute to its transactivation function and promoter specificity. A specific combination of posttranslational modification of p53 may result in the transactivation of a specific p53 target gene. For example, methylation of lysine (K372) by methyltransferase Set7/9 promotes p53 activity, in particular the transactivation of p21 [29]. p53 modifications by SUMO and Nedd8 also occur on its carboxylic terminal. SUMOylation of p53 contributes to its transcriptional activity [28]. Regulation of cell cycle and apoptosis are the primary functions of p53, functions that are achieved through its various transcriptional target genes.

1.2.2 Cell cycle arrest

Cellular stress signals that activate p53 pathway transduce signals predominantly culminating in cell cycle arrest or apoptosis. The choice made between the two does not entirely rest upon p53, but the many variables such as, the type of stress signal and the cellular background, which includes co-factors necessary for the initiation of transcriptional of p53 target genes. The ratio between expression levels of pro-apoptotic and pro-survival cellular proteins is also a deciding factor between cell survival (cell cycle arrest) and death (apoptosis).

Replication of DNA and retaining its integrity is fundament for a successful cell division. The events leading to DNA replication for the generation of two daughter cells are grouped into different stages,

G0 phase - Cells have exited cell cycle and do not divide

G1 phase - Checkpoint phase where cells are committed/prepared to enter cell cycle

S phase - DNA synthesis/replication phase

G2 phase - Checkpoint phase where cells continue to grow and prepare for cell division

M phase - Nuclear division, followed by cellular division resulting in two daughter cells

Cell cycle checkpoints are mechanisms that ensure the timing of various events related to DNA replication during cell division. Cyclins in complexes with Cyclin Dependent Kinases (CDKs) regulate the progression of cell cycle. p53 induced cell cycle arrest is primarily through its well established transcriptional target p21 (WAF1/CIP1) [30], that induces arrest of cells in the G0/G1 or the G2 phase cell cycle [31]. p21 is a CDK inhibitor which by forming a complex with CDK1 or CDK2 is able to induce arrest of cells in G0/G1 or the G2 phase of cell cycle [32, 33].

Another pathway leading to cell cycle arrest as a response to DNA damage is through p53 induced 14-3-3-sigma that leads to the arrest of cells in the pre mitotic (G2/M) phase of cell cycle [34]. DNA damage induced in human colorectal cancer cells lacking 14-3-3-sigma was only briefly able to exit cell cycle, whereas those expressing the protein were arrested in the G2/M phase. The inability to sequester CyclinB1/cdc2 complex as they lacked 14-3-3-sigma allowed these cells to enter mitosis resulting in death, referred to as ‘mitotic catastrophe’[35]. Ectopic expression of 14-3-3-sigma in colorectal cancer cell lines resulted in cell cycle arrest in the G2 phase, with DNA content of 4N. However, prolonged expression of 14-3-3sigma in these cells resulted in polyploidy [34].

In addition to DNA damage response, p53 is also activated in response to limited availability of nutrients or a deregulated pathway sensing the availability of nutrients. Under normal conditions of adequate glucose and amino acids levels, signals through the IGF-1/AKT/mTOR pathways allow cell growth and division. However, when cells are under nutritional stress, they undergo senescence or quiescence. Prolonged nutrient deprivation can

also lead to cell death. Through modulating the expression of IGF-BP3, PTEN, TSC2, AMPK b1, Sestrins1/2 and REDD1 by p53, the IGF-1/AKT/mTOR pathways are negatively regulated, thus altering cellular metabolism [36].

1.2.3 Apoptosis

The mechanism of cell death by apoptosis was discovered while studying the early development of the organism, *Caenorhabditis elegans* [37]. The organism develops from an initial 1090 somatic cells, of which 131 cells undergo 'programed cell death' at specific time points during the course of development. The invariant number and time of cell death during the course of development of the worms was realized to be vital for the development of the organism.

The tumor suppressor function of p53 is to maintain tissue homeostasis, controlling the number of cells in a particular tissue/organ system. Prevention of the aberrant proliferation of cells is one of the regulatory mechanisms of establishing homeostasis. Another crucial way of maintaining homeostasis is through the regulation of cell number through a well choreographed mechanism of programmed cell death – apoptosis [38]. Loss of p53 function has been associated with about half of the cancer incidences, providing cancer cells with the advantage of impaired apoptosis following cellular stress signals. Cell death through apoptosis follows a well orchestrated sequential mechanism elicited through a number of molecular pathways, the most characterized and prominent of which are the extrinsic/death receptor pathway and the intrinsic/mitochondrial pathways.

Cells that receive signals for apoptosis undergo certain fundamental biochemical changes that contribute to programmed cell death. The proteolytic cleavage of caspases, which in normal cells are expressed as inactive proenzymes is one of the important changes in cells undergoing apoptosis. Proteolytic cleavage of the proenzyme to form active caspases amplifies the apoptotic signaling pathway leading to the rapid death of cells. Caspases themselves have proteolytic activity, sequentially cleaving other caspases and culminating with the fragmentation of DNA and eventually death [39].

1.2.3.1 Extrinsic pathway of apoptosis

The cell in this case receives extracellular signal to initiate apoptosis. The extracellular domains of 'death receptors' are the docking sites for the death signal (ligand) to be transmitted to the intracellular components triggering a cascade of events leading to cellular death. FasL/Fas, Apo3L/DR3, Apo2L/DR4, Apo2L/DR5 and TNFa/TNFR1 are well characterized pairs of ligands and their corresponding death receptors [40-43]. The mechanism of extrinsic apoptotic pathway has been well defined in the FasL/Fas model. As a first step, Fas trimerises following the binding of it by the FasL, initiating the downstream apoptotic machinery. The Fas associated protein with death domain (FADD), an adaptor protein is recruited to the cytoplasmic death domain of the Fas receptor. Consequently, FADD recruits procaspase 8, bridging Fas and procaspase 8 to form the death inducing signaling complex (DISC). The formation of the DISC domain results in the autocatalytic

activation of procaspase 8 [40]. With the activation of caspase 8, execution phase of apoptosis is triggered.

1.2.3.2 Intrinsic pathway of apoptosis

Non-receptor mediated apoptotic stimuli target molecules in the cell that are associated with the destabilization of the mitochondria. Stimuli for intrinsic pathway may oppose cell survival or favor apoptosis. Deprivation of nutrition, growth factor and cytokines are negative signals that breakdown cell survival programs. On the contrary, positive signals that favour apoptosis are stimuli associated with DNA damage, hypoxia, toxins, free radicals and infectious agents. These stimuli destabilize the mitochondrial trans-membrane potential leading to the release of caspase activating proteins into the cytoplasm. This early phase of apoptosis leads to the formation of 'apoptosome', a complex formed between the cytochrome c released from the inner wall of the mitochondria, procaspase-9 and Apaf-1 [44].

Cells have mechanisms to counter act apoptosis initiated through the intrinsic pathway. Inhibitors of apoptosis proteins (IAP) are pro-survival proteins that block the function of caspases [45]. However, IAPs can also be inhibited through the formation of complexes with the pro-apoptotic Smac and HtrA2, also released from the mitochondria after pro-apoptotic stimuli, allowing caspases to amplify the downstream apoptotic signals [46, 47]. Following release of cyt c from the mitochondria, apoptosis inducing factor (AIF), caspase activated DNAs (CAD) and endonuclease - proteins that are responsible for DNA condensation and fragmentation are also released [48].

Bcl-2 family of proteins regulate the events leading to apoptosis involving mitochondria [49]. The Bcl-2 family consists of both pro-survival/anti-apoptotic and pro-apoptotic proteins. By regulating the expression of Bax - a member of Bcl-2 family of proteins, p53 plays a critical role in the induction of apoptosis through the mitochondrial pathway [50, 51]. p53 also positively regulates the expression pro-apoptotic Puma, Noxa and Bid - members of 'BH3 only' class of Bcl-2 family proteins. The BH3-only proteins act upstream of Bax and facilitate the destabilization of the mitochondrial membrane potential to induce apoptosis. In addition, p53 can control apoptosis involving mitochondria in a transcriptionally independent manner. In irradiated mouse thymocytes, p53 itself translocates to mitochondria, binds to the anti-apoptotic Bcl-XL and Bcl-2 proteins leading to the release of cyt c into the cytoplasm [52]. Thus, the balance between the levels of pro-apoptotic Bcl-2 proteins and anti-apoptotic Bcl-2 proteins plays a critical role in determining the fate of the cell, the release of cyt c from the mitochondria into the cytoplasm and the subsequent activation of procaspase 9 leading to apoptosis.

1.2.3.3 Execution pathway of apoptosis

Both, extrinsic and intrinsic pathways converge at the execution pathway to follow the final events of apoptosis. Caspase-3/6/7 function as primary effector caspases in the execution pathway [53]. Activation of the effector caspases leads to the cleaving of PARP, NuMA, cytokeratins and other proteins that are responsible for the characteristic morphological and

biochemical changes observed in apoptotic cells [54]. Activation of caspase-3 through the initiator caspases – 8/9/10 specifically activates CAD, which otherwise would be inhibited by complexing with its inhibitor, ICAD. Activation of CAD results in the degradation of chromosomal DNA [53]. Caspase-3 cleaves gelsolin, an actin polymerization protein and thus leading to the formation of apoptotic bodies through cytoskeletal disintegration. The process of apoptosis culminates by the massive externalization of phosphatidylserine present in the cell membrane's lipid bilayer. The apoptotic cells with exposed phosphatidylserine molecules are recognized by phagocytic cells to be cleared off by endophagocytosis.

Thus, p53 dependent apoptosis is mediated through the induction of its downstream pro-apoptotic target genes Bax, Puma, Noxa and Fas. However, recent studies has shown that apoptosis and cell cycle arrest are dispensable functions for p53 mediated tumor suppression [55], and the expression of Puma, Noxa and p21 are unessential for p53 mediated tumor suppression [56]. This suggests other p53 transcriptional targets and mechanisms such as, metabolism to be involved in p53 mediated tumor suppression.

1.2.4 Post-transcriptional gene regulation

The most well defined and commonly referred mechanism of gene regulation by p53 is through its ability to bind to DNA and activate transcription. p53 can also indirectly regulate gene expression at the post-transcriptional level through the induction of specific target genes that regulate the stability of target mRNA. Zink finger domain containing Wig-1 is one such p53 target [57, 58] that can regulate the stability of certain mRNAs by binding to site specific AU-rich region on the 3'UTR [59]. Wig-1 has been shown to regulate p53 mRNA by a positive feedback loop mechanism. Binding of Wig-1 to the AU-rich 3'UTR of p53 mRNA stabilizes it by preventing its deadenylation. This Wig-1 mediated stabilization of p53 mRNA is reflected on the increased p53 protein levels and its response to DNA damage. Other 3'UTR binding proteins like, human antigen R (HuR) [60] and ribosomal protein L26 (RPL26) [61] have also been shown to stabiles p53 mRNA and enhance its translation.

mRNA stability is also regulated by short non-protein coding micro RNAs (miRNAs) which are incorporated into a RNA-induced silencing complex (RISC). As the name suggests, the miRNAs negatively regulate gene expression by forming a complex with the target mRNA and subjecting it to mRNA degradation. p53 is known to induce the miR-34 family consisting of miR-34a, b and c [62-65]. The miR-34 family was shown to repress tumor growth and metastasis through inhibition of genes contributing to cancer progression, EMT, metastasis and stemness of cancer cells (examples). In the immunological context, p53 dependent expression of miR-34a is essential for the repression of IL-6 receptor, thus inhibiting the STAT3 signaling [66].

1.2.5 p53 in immune system

p53 knockout mice have been a very useful animal model to study the contribution of p53 to various biological processes and the damage done to the system in its absence. Such mice are prone to develop T cell lymphomas in spite of an apparent normal T lymphocyte

development and immune response [67, 68], underlining p53's role in regulating T cell proliferation. Also, CTLs from p53 mutant mice expanded more efficiently than from p53 wild type mice [69], again indicating a role for p53 in regulating T cell proliferation. Similar observations were made in p53 silenced humanized mouse model, normal T cell development and a normal immune response to antigen stimulation. However, upon prolonged antigen stimulation, p53 silenced T cells showed a significant growth advantage over T cells expressing p53 [70].

Despite studies in p53 knockout mice that showed that p53 loss has no significant influence on the T cell development and function, recent studies suggest p53 regulates genes involved in the immune response. p53 has been shown to regulate IL6 expression [66] and polarization of macrophages to M2 [71].

1.3 T lymphocytes

Bone marrow is the site of generation of the common lymphocyte progenitor cells. These cells migrate to the thymus where they rearrange their TCR genes [72], mature and undergo differentiation to give rise to the two major subtypes of T cell, CD3+ CD4+ and CD3+ CD8+. T cells are among the most versatile cell in the body. The complex T cell receptor (TCR) they express on the cell surface recognizes its cognate ligand, an antigen peptide bound to major histocompatibility complex (MHC) class I or class II [73]. Those T cells that recognize MHC bound self-antigens are self-reactive and are eliminated by apoptosis, a process called negative selection. T cells whose TCR fail to bind peptide bound MHC also undergo apoptosis, a process called death by neglect [74]. T cells that survive differentiate into either CD4+ or CD8+ cells.

T cells have diverse but distinct functions based on their state of differentiation in the peripheral immune system.

CD4+ T cells are highly divergent in their differentiation, function and cytokine profile [75]. CD4+ T cells help B cell maturation, activation and antibody production. They also activate cytotoxic T cells or they suppress the immune reaction. The following CD4+ T cell subsets have been identified and characterized.

T helper type 1 (Th1) cells: IL12 and INFγ secreted by dendritic and NK cells initiate the differentiation of Th1 cells [76]. INFγ produced by Th1 cells inhibit the development of Th2 and Th17 cells [77]. Th1 cells are important for host defense against viral and intracellular bacterial pathogens.

T helper type 2 (Th2) cells: IL4 and IL2 are critical for Th2 cell differentiation [78]. IL6 produced by APCs also promotes Th2, but inhibits Th1 lineage differentiation [79]. Th2 cells are important for host defense against extracellular pathogens and are also important for allergic responses.

T helper type 9 (Th9) cells: Subset of Th2 cells that differentiate to IL9 producing Th9 cells induced by IL4 and TGFβ [80]. Abnormal increase in Th9 cells is associated with allergic inflammation [81] and autoimmune encephalitis [82].

T helper type 17 (Th17) cells: IL6, IL21, IL23 and TGFβ are essential for the differentiation of Th17 cells [83]. IL17A and IL17F are the major cytokines secreted by Th17 cells [84]. This T cell subtype has been implicated in pathological and protective role during inflammation.

T helper type 22 (Th22) cells: This subset of CD4+ T cells are recruited to skin where they defend against microbial pathogens, but are also associated with inflammatory skin disorders. These cells secrete high levels of IL22 and INFα [85].

Follicular helper T (Tfh) cells: These cells are involved in the regulation and development of antigen-specific B cell immunity. The surface markers for Tfh cell identification are CXCR5 along with ICOS, PD-1. They also express high levels of SAP. Tfh cells are localized in the follicles of lymph nodes and express BCL6 [86].

Regulatory T (Treg) cells: Treg cells are characterized as CD4+ CD25+ cells that express FoxP3. They comprise 5-10% of the total CD4+ cells and negatively regulate innate and adaptive immune responses [87]. These cells are responsible for maintaining immune homeostasis via inhibition of differentiation and activity of pro-inflammatory T helper cells. FoxP3 expression is the most commonly used marker for Treg cells in mice and humans. A specific subtype of regulatory cells has been described in the germinal center as well, Tfr, where Foxp3 is expressed in cells that carry the markers of Tfh cells: CD4+ CXCR5hi PD1hi. Human Treg cells are further differentiated by low expression levels of CD127 [88].

Natural killer T (NKT) cells: These cells mature in the thymus but also express NK lineage receptors. They are immunomodulatory CD1d restricted T lymphocytes that recognize lipid antigens [89].

CD8+ cytotoxic T lymphocytes (CTLs): These T cells express an alpha beta T cell receptor (TCR) and execute the direct killing of virus infected, damaged, and tumor cells. CTLs most commonly produce IFNγ following TCR stimulated activation. The cytotoxic function of CTLs is accomplished by establishing physical contact with the target cells, followed by Perforin mediated pore formation and the release of the serine protease granzyme B into the target cells. In the target cell granzyme B activates procaspase 3 and Bid to trigger the intrinsic apoptotic pathway. The population of activated CTLs predominantly consists of short-lived effector cells, which die rapidly after the infection has been cleared, but a small group of antigen specific effector cells make up the memory cell population [90].

T cell survival is influenced by the signals the cell receives through the TCR complex, CD4 or CD8 co-receptors and co-stimulatory molecules (including CD28), adhesion molecules and cytokines (such as interleukin 2 (IL2), IL13 and IL15) [91]. Proliferation and resistance towards T cell death can be regulated by several cytokines like, IL2, IL4, IL7, IL15 and IL21

[92]. Among the ILs, IL7 is an important pro-survival signal for naïve T cells. IL7/IL7Ra interactions result in activation of STAT5 and delayed but sustained AKT activation, these signaling pathways are critical for IL7-mediated uptake of glucose [93].

1.3.1 Regulation of lymphocyte population

The immune system plays a critical role in safeguarding the host against pathogens. Cells of the immune system function with a great degree of specificity directed towards a particular antigen. In response to an antigen, naïve immune cells are sensitized to bring about changes in morphology and cellular functions to generate effector cells. The effector cells by themselves or with aid from other cells counteract the pathogens or the pathogen infected cell/s. The engagement of specific antigen receptors on cell surface triggers the process of activation. Activation of immune cells is followed by their increased proliferation and/or motility and execution of their effector functions. Following a successful immune response, the immune system starts to shutdown, characterized by the dramatic decrease in the number of activated cells. This shutdown of the immune system is facilitated by the cessation of proliferation and apoptosis of activated cells. This process also promotes the restoration of resting state, thus establishing homeostasis.

The shutdown of T cell response is brought about by apoptosis of activated T cells, allowing the decline in its number (contraction phase). Activation induced cell death (AICD) and activated T cell autonomous death (ACAD) are two concepts that explain homeostatic regulation of T cell immune responses.

1.3.1.1 Activation induced cell death (AICD)

The most well characterized mechanism of apoptosis in activated T cells is through the interaction of FasL (APO-1L) and its corresponding Fas receptor (CD95) [42]. Mouse models with defective FasL/Fas genes have been shown to have defective apoptotic mechanism to regulate T cell population leading to lymphoproliferation and autoimmunity [94, 95]. Interaction of the FasL/Fas induces the formation of DISC, composed of trimearised Fas, FADD and two procaspases-8 and 10. Formation of DISC helps the activation of these procaspase that will initiate the progress through the apoptotic pathway. In this sequence of events the initiator caspases-8 and 10 are critical to transmit the apoptotic signal downstream to the effector caspases [96].

The apoptotic signal initiated through the activation of Fas receptor and transduced through the formation of DISC also relies on the destabilization of mitochondria to amplify the apoptotic signals downstream. The cross talk between the receptor dependent apoptosis and the mitochondria dependent intrinsic apoptotic pathway is mediated through the caspase-8 cleavage of Bcl-2 family member-Bid, giving rise to truncated Bid (tBid) [97]. The mitochondrial membrane is destabilized by tBid, releasing cytochrome c into the cytosol. This facilitates the formation of apoptosome and activation of caspase-9, which in turn activates the effector caspases, culminating in apoptosis [98].

1.3.1.2 Inhibition of AICD

Fas expression is upregulated with the activation of T cells indicating that T cells are programing themselves for apoptosis very early during an immune response. However, during activation and expansion phase of the immune response T cells evade apoptosis induced through Fas by regulating the level of activated caspases8/10. This regulation of caspases during early stage of activation is brought about by the competitive binding of cellular FLICE-inhibitory protein (cFLIP) to FADD molecules [99]. The ratio between procaspases and cFLIP available to bind to FADD determines the initiation of apoptosis. The binding of cFLIP to FADD has been implicated in the negative regulation of AICD [100]. Several isoforms of cFLIP have been reported of which cFLIP long and short ($cFLIP_L$ and $cFLIP_S$) have been associated with the inhibition of AICD [101]. The expression of c-$FLIP_S$ is strongly upregulated upon initial T cell activation. Co-stimulation of T cells via CD28 elevates $cFLIP_S$ expression providing resistance to apoptosis during the early stages of T cell activation, thus facilitating clonal expansion [102]. With the decline of $cFLIP_S$ expression, Fas dependent AICD is initiated as procaspase-8/10 molecules are now able to bind to FADD enabling the formation of DISC. The mitochondrial apoptotic pathway is also inhibited during the initial T cell activation and expansion through the action of $cFLIP_S$ and the anti-apoptotic Bcl-X_L. The mitochondria dependent apoptotic machinery is inhibited through the binding of Bcl-X_L to the pro-apoptotic Bcl-2 proteins. Thus through the expression of $cFLIP_S$ and Bcl-X_L during the initial phase of activation and expansion, T cells resist Fas mediated apoptosis.

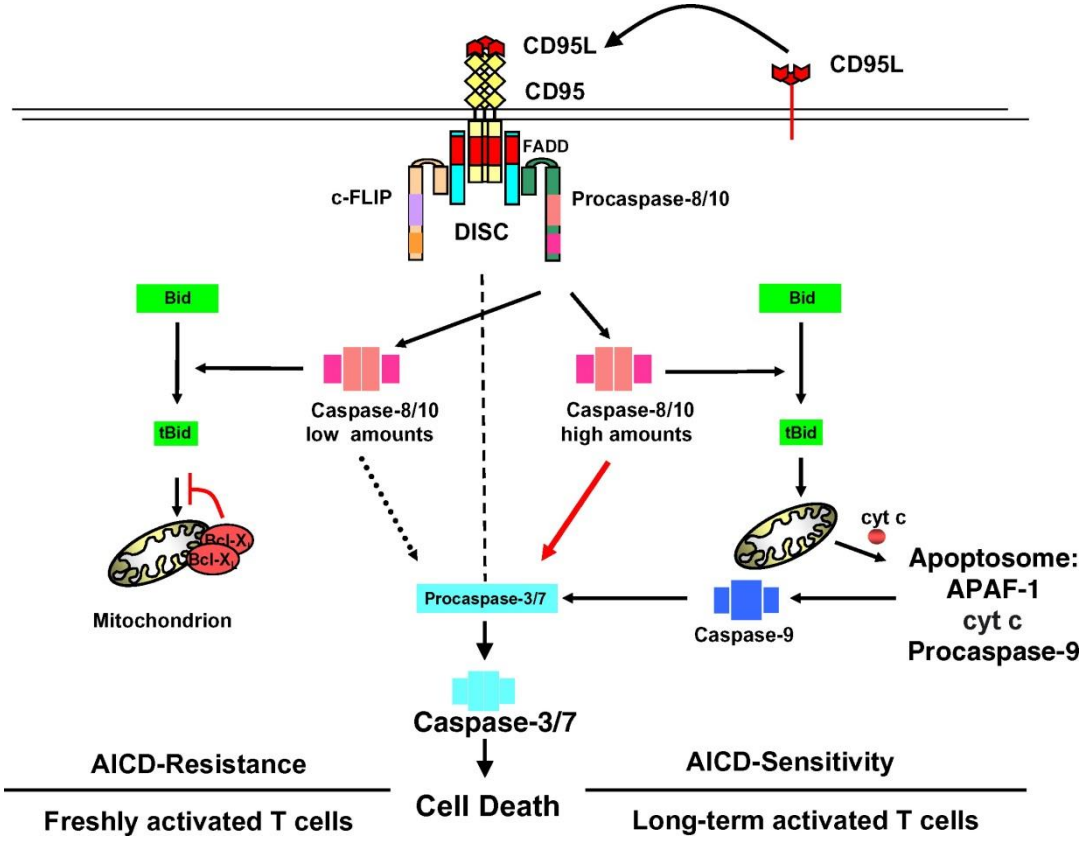

Figure: Activation induced cell death pathway [109].

1.3.1.3 Activation cell autonomous death (ACAD)

In absence of the appropriate survival signals (i.e. cytokine depravation), activated T cells are subjected to ACAD, that is apoptosis independent of the cell death receptor pathway [103]. Under such circumstances expression of pro-apoptotic Bcl-2 family proteins BIM and PUMA increases and they induce ACAD through the intrinsic apoptotic pathway that destabilizes the mitochondrial integrity [104, 105]. It has been reported that during the immune contraction phase BIM expression levels are elevated. Binding of BIM to pro-survival Bcl-2 will allow the release of cyt c through the pro-apoptotic function of Bak and Bax. Understanding precise mechanism of ACAD *in vivo* is of great interest. It is postulated to play a significant role in regulating T lymphocyte homeostasis during low antigen load, while AICD regulates T cell deletion during high antigen load.

1.3.1.4 Cytokine mediated regulation of T cell survival

T cell survival is also influenced through a number of cytokines. IL2 is an essential cytokine for activated T cells that helps proliferation, thus the establishment of a protective immune response [106]. Naïve T cells that are resistant to AICD, produce IL2 following activation and also express its high affinity IL2Rα (CD25) [107]. IL2, through the PI3K pathway, can induce the expression of antiapoptotic Bcl-2 proteins, protecting the cells from apoptosis. However, studies in mouse models deficient in IL2 or CD25 show impaired AICD, suggesting the requirement of IL2 for effective AICD [108]. The increase in FasL and decrease in c-FLIP expression at the end of the expansion phase has been postulated to be IL2 dependent.

IL15 is another cytokine that shares the same IL2α receptor and it also stimulates T cell proliferation. However, IL15 can inhibit IL2 dependent sensitization for AICD [109].

IL7 is crucial for T cell development, deficiency of which is associated with the development of severe combined immunodeficiency (SCID). IL7rα deficiency has been associated with low expression of pro-survival Bcl-2 proteins, leading to impaired T cell survival [110].

1.4 Epstein-Barr virus (EBV)

Human herpesvirus 4, Epstein-Barr virus (EBV) is a gammaherpesvirus, leading to one of the most common viral infections in humans, with around 90-95% of adult population being infected. EBV is a double stranded DNA virus. It was first identified while studying Burkitt lymphoma (BL), a B cell lymphoma [111, 112]. Following the discovery of EBV, its ability to transform B cells *in vitro* was established [112, 113]. This *in vitro* system of transformation was exploited to study the mechanisms of EBV induced B cell transformation and the immune response mounted against it. Based on the *in vitro* findings, EBV was considered to be the driving force behind the mechanism in the development of BL. However, the discovery of EBV negative BL which shared similar cytogenetics and pathology with EBV positive BL, suggested that EBV was not the primary cause of BL, but likely contributes to its pathogenesis.

EBV preferentially infects B lymphocytes and epithelial cells. The viral gp350 and gp42 proteins bind to CD21 and HLA class II molecules on B cells and facilitates entry of EBV into the cells [114, 115]. Epithelial cells do not express CD21, here the fusion occurs through the viral BMRF-2 and gH/gL envelop proteins binding to β1 and αvβ6/8 integrins respectively [116]. Primary EBV infection most often occurs during the early childhood and is asymptomatic. If the primary infection is delayed, about half of the infected individuals develop a self-limiting lymphoproliferative disease called infectious mononucleosis (IM) or more popularly known as the kissing disease. IM is typically characterized by lymphocytosis of CD8+ T lymphocytes. Following the recovery from IM, EBV continues to persist in the infected host for the rest of the individual's life as an asymptomatic infection [117].

1.4.1 Type III latency

In vitro infection of B cells with EBV establishes a latent infection giving rise to transformed lymphoblastoid cell lines (LCLs). Six EBV nuclear antigens (EBNA1-6) and three membrane associated proteins (LMP1, LMP2a and LMP2b) were characterized in LCLs. In these cells, the virus uses one of its two promoters (Wp or Cp) to generate a single giant mRNA. The mRNAs for the six EBNAs are spliced from this giant mRNA. EBV gene expression of this pattern is called the growth program – type III latency. The virus also encodes noncoding RNAs, EBER1&2, BanHI-A rightward transcripts (BARTs) and miroRNAs. The Bcl-2 homologs, BALF1 and BHRF1 are transiently expressed during the early stage of primary infection [118]. The virus encodes 23 well characterized miRNAs that are expressed in two clusters: the BART cluster that encodes 20 miRNAs, highly upregulated in latently infected epithelial cells and low levels in B cells. BHRF1 is the other cluster encoding 3 miRNAs, upregulated in type III but not detectable in type I/II latent B or epithelial cells. The expression of viral proteins and miRNAs interacts and influences various host proteins, miRNAs and thus cell signaling mechanisms. Of all the viral proteins expressed, EBNA-2, 3, 5, 6, LMP-1, BALF1 and BHRF1 are essential for *in vitro* B cell transformation.

The EBV genome is linear in the virion, but following infection of B cells it is circularized and maintained as an extra chromosomal circular structure called, episome. EBNA-1 is critical for maintaining the EBV episome. It binds to the origin of plasmid replication (oriP) and anchors it to the host chromosomes during mitosis, thus duplicating and partitioning the viral genome during cell division [119]. In addition to its role in maintaining the viral episome, EBNA-1 has also been shown to play an anti-apoptotic role in EBV carrying malignant cells [120].

EBNA-2 is the first viral protein expressed following infection [121]. It contributes to the establishment and maintenance of type III latency through the activation of LMP-1, -2 and Cp promoters. It trans-activates the through hijacking the Jκ recombination signal binding protein (RBP-jκ), the major transcriptional factor involved in Notch pathway [122-124].

EBNA-5 is among the first viral proteins along with EBNA-2 to be expressed following B cell infection [125]. Forming a trimolecular MDM2-p53-EBNA5 complex, the transactivation function of p53 is abrogated [126].

LMP-1 is a glycoprotein that acts as a constitutively active CD40. It activates the NF-κB, MAP kinase, phosphatidylinositol 3-kinase (PI3K) and ERK-MAPK signaling pathways [127, 128]. With the engagement of multiple signaling pathways, LMP-1 expression induces expression of anti-apoptotic proteins (cFLIP, cIAP1 & 2, Mcl-1) that contributes to the survival of EBV infected B cells [129, 130]. Genes involved in cellular adhesion (ICAM-1, SLAM, CD40, CD48, LFA-1 & 3) and also those that are associated with metastasis (VEGF, Cox-2, MMP-1 & 9) are induced with the expression of LMP-1 [131]. On the contrary, E-cadherin and BCL6 are repressed by LMP-1 [132-134]. Thus LMP-1 is a major transforming protein of EBV required for efficient B cell immortalization. Studies on transgenic mice have confirmed that LMP-1 mimics an active CD40 receptor and aids B cell activation and differentiation [135]. B cells of mice lacking CD40 receptor but expressing LMP-1 demonstrated ability to proliferate, enhanced expression of activation antigens and underwent extrafollicular B cell differentiation. However, LMP-1 blocked germinal center (GC) formation even in the presence of CD40. This inhibition of CG formation is through LMP-1 mediated repression of BCL6 [127]. Expression of LMP-1 in BL cells inhibited proliferation plausibly through the suppression of BCL6.

1.4.2 Type IIa latency

In this EBV gene expression pattern, only one nuclear antigen EBNA-1 along with LMP-1 and -2 are expressed. This pattern of EBV gene expression was first observed in nasopharyngeal carcinoma (NCP) [136, 137]. Other malignancies associated with type IIa latency are, the classical Hodgkin lymphoma, EBV positive T and NK cell lymphomas. Unlike in type III latency where the transcription of EBNA mRNAs originate from Cp or Wp, in type II latency a different promoter, BamHI Q-region (Qp) drives the exclusive transcription of EBNA-1 only [138-140]. Also, the expression of LMP-1 and -2 are differently regulated due to the lack of EBNA-2 expression.

1.4.3 Type IIb latency

This special latency program was identified in B-chronic lymphocytic leukemia (B-CLL) cells infected *in vitro* with EBV [141]. It expresses all six nuclear proteins, EBNA1-6, but lack LMP-1 expression [117]. The *in vitro* infection of B-CLLs with EBV does not yield LCL, consistent with the requirement of the expression of LMP-1 for B cell immortalization [142]. EBV is not involved in the pathogenesis of B-CLL *in vivo* and *in vitro* occurrence of EBV positive B-CLL clones are also extremely rare. The lack of LMP-1 despite EBNA-2 expression in the type IIb latency is intriguing, but there is still no explanation for it. This type of latency program has been reported not only in *in vitro* infected B-CLL cells, but it has been detected by immunohistochemistry in lymphoid tissues of PTLD, IM patients and EBV infected humanized mouse models as well [117].

1.4.4 Type I/0 latency

This is the 'EBNA-1 only' expression patter of EBV. Similar to the type II latency ENBA-1 transcription is driven by the Qp [143]. Endemic BLs are a classic example of type I latency program. EBNA-1 is essential for the replication of the viral eposome and its distribution to daughter cells during mitotic division [144, 145]. EBV carrying B cells that are not subjected to mitotic division express the non-translated viral RNAs, the EBERs. This viral latency program where only the EBERs are expressed is classified as type 0 latency.

1.4.5 T lymphocyte response to EBV infection

In most cases, EBV infection is not life threatening as it is effectively controlled by the host immune response [146]. In response to EBV infection both the humoral and cellular immune systems are activated. Antibodies generated against the viral antigens are exploited for the diagnoses of the infection.

The HLA class I restricted CD8+ T cell response to EBV infection during IM is the most profound and important immune compartment regulating the infection [147]. Early studies of CD8+ response to EBV employed the *in vitro* system of challenging PBLs to autologous LCL (latency III) in order to generate CD8+ T cell lines specific for EBV antigens. From these studies, the identified epitopes were most often derived from EBNA-3, -4 or -6 and less often from other latent proteins, but least likely from EBNA-1 [148, 149]. A glycine-alanine repeat domain in EBNA-1 was identified to protect it from proteasomal degradation for MHC class 1 processing [150]. However, several class II restricted EBNA-1 peptides have been identified *in vivo* studies on IM cases showing that half of the CD8+ response is highly focused against proteins of the early lytic cycle – BZLF1, BRLF1, BMLF1 and BARF1 [151-154]. It was also observed that in most individuals CD8+ response was generated against more than one EBV antigen.

Very early studies of HLA class II CD4+ cell response to EBV during IM reported no or very little increase in their numbers [155]. However, with the development of HLA class II tetramer techniques it has been possible to identify subtle bursts of EBV specific CD4+ T cell response [156], although not matching the CD8+ T cell response neither in size nor in activity. Studies have shown that both lytic and latent proteins released from infected B cells were cross-presented by neighboring antigen presenting cells to be recognized by CD4+ T cells. However, unlike the CD8+ T cell responses, the CD4+ T cell response leaned more towards latent than lytic antigens [157]. EBNA-1 specific CD4+ T cells were found more frequently in virus carrying healthy individuals but barely detectable in acute IM [152, 158], reiterating EBNA-1's poor ability to be processed and presented by antigen presenting cells.

1.4.6 EBV's way to memory B cells

It is well established that once infected, EBV is harbored in memory B cells for the rest of the individual's life. The frequency of EBV infected B cells in the blood of IM patients range from 1in 2 to 1 in 10^2 memory B cells [159]. However, in healthy carriers the frequency of

EBV carrying B cells is far much lower, ranging from 1- 50 in 10^6 B cells [160]. Type III latency, similar to the gene expression pattern LCL is found during IM, but such cells owing to their high immunogenicity are efficiently eliminated by the cellular immune responses.

Studies of on EBV positive tonsilar B cell subpopulations reveled that, type III latency pattern was restricted to the naïve B cell population, while type II latency was restricted to B cells in the GC [161]. EBV carrying memory B cells isolated form blood express type I/0 latent viral gene expression with no detectable LMP-1 mRNA, but very low LMP-2 mRNA. Thorley Lawson postulated a theory on how EBV gains access to the memory B cell pool [162]. According to this theory – the majority of the EBV infected naïve B cells expressing the type III pattern are successful eliminated, while those that escape immunesurveillance enter GCs and differentiate into GC B cells. These EBV carrying GC B cells express the type IIa latency-EBNA-2 and LMPs. As LMP-1 and LMP-2 mimics CD40 and BCR signaling pathways respectively, it is postulated that the signals essential for survival and differentiation of EBV carrying GC B cells (type IIa latency) are provided by these proteins. The model further postulates that the number of EBV encoded proteins will be further reduced in EBV harboring B cells upon leaving the GC environment. Once cells are out of the CG environment, the EBV infected resting memory B cells will express only the EBERs (type I latency). EBNA-1 expression is induced upon the stimulation of EBV positive memory B cells to enter cell cycle. Through the induction of EBNA-1, the viral episome is replicated and carried by the daughter cells. When EBV carrying memory B cells differentiate to plasma cells, EBV's lytic replication is initiated to produce new virus progeny.

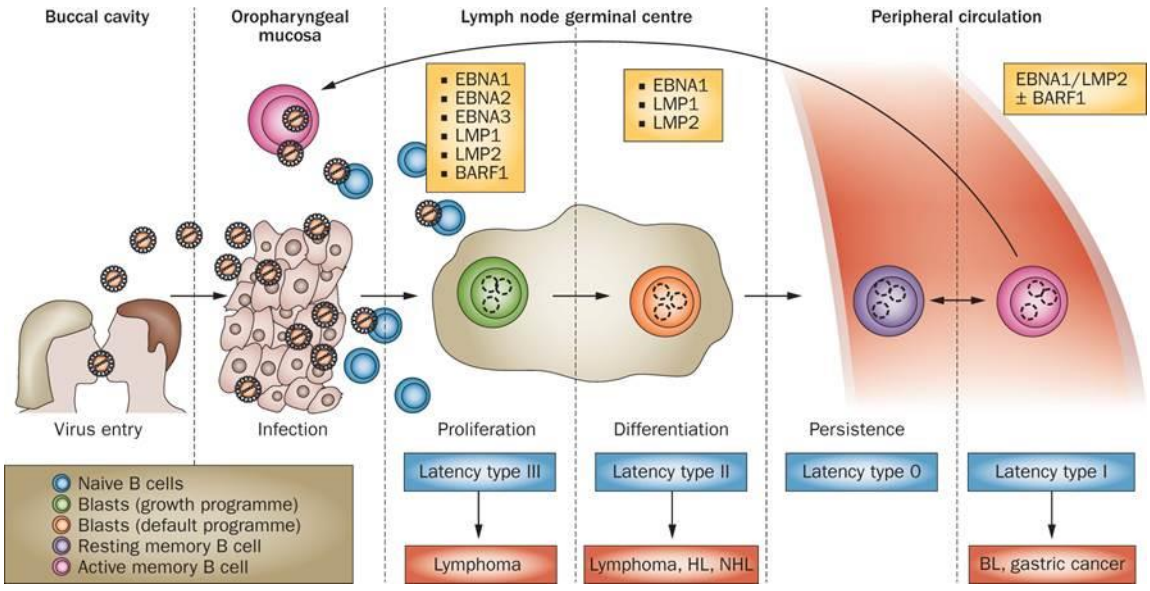

Figure: EBV infection and latencies [253].

1.4.7 The X linked lymphoproliferative disease (XLP)

XLP is a rare immunodeficiency which is the result of mutations or the deletion of the SH2D1A/SAP (SLAM associated protein) gene [254-256]. SAP is expressed in T and NK cells, it binds to and modulates signals initiated through SLAM family of surface receptors. Individuals suffering from the XLP disease are selectively susceptible to EBV infection but not to other herpes virus infection. Primary EBV infection in XPL individuals results in uncontrolled T and B cell proliferation. 50% of XLP patients die as a consequence of primary EBV infection - fatal IM, the major manifestation of XLP which is caused by overwhelming T cell activation accompanied by impaired AICD and loss of function. The unchecked proliferation of T cells (caused by the loss of SAP's pro-apoptotic function) is associated with extensive necrosis in the liver, bone marrow and other organs, thus resulting in fatality. CD8+ T cell response to EBV transformed B cells is defective in XLP patients [257, 258]. SAP deficient CD8+ T cells exhibit impaired cytotoxicity to antigens presented by B cells. However, the cytotoxicity exhibited by SAP deficient CD8+ T cells was similar to CD8+ T cells expressing functional SAP, when the same antigens were presented by other antigen presenting cells. This impaired function of SAP deficient CD8+ T cell fails to eliminate the EBV infected B cells [259]. Deficient SAP in Tfh cells affects the impending differentiation, affinity maturation and class switch recombination of B cells in the GC, leading to dysgammaglobulinemia seen in XLP patients [260]. The high incidence of malignant lymphomas (40%) manifested in XLP patients is also likely the consequence of deficient pro-apoptotic function of SAP [241, 242].

1.5 Multiple myeloma (MM)

MM originates from an asymptomatic premalignant proliferation of monoclonal plasma cells. Multistep genetic aberrations accompanied by changes in the microenvironment lead to the transformation into a malignant neoplasm. Multiple myeloma is a heterogeneous disease understood to evolve from a monoclonal gammopathy of undetermined clinical significance (MGUS) [163]. Unlike CML, a single driver mutation is not responsible for the manifestation of the disease, it exhibits a number of genetic abnormalities. However, the primary early chromosomal translocation in MM is the immunoglobulin switch region on chromosome 14 (q32.33) juxtaposed to proto-oncogene MAF (t[14.16][q32.33;23]) and MMSET on chromosome 4p16.3 [164, 165]. The secondary, late-onset translocations and gene mutations that are implicated in the pathogeneses of the disease progression include complex karyotypic abnormalities in Myc, activation of N-Ras, K-Ras, mutations in FgFr3, p53 and the inactivation of CDKN2A and CDKN2C [166, 167].

Genetic abnormalities and chromosomal alternations adversely influence the expression of adhesion molecules and response to growth stimuli in the microenvironment. MM cells greatly rely on cellular and non-cellular components of the tumor microenvironment like, bone marrow stromal cells, endothelial cells, IL6, insulin like growth factors and vascular endothelial growth factors for their survival and growth [168]. Among these, IL6 is known to play an important role through the activation of STAT2/STAT3 pathway leading to increased

levels of anti-apoptotic Bcl-Xl and Mcl1, thus promoting survival and resistance to drug induced apoptosis of MM cells [169, 170].

Proteasomal inhibitors and immunomodulatory drugs can target the disruption of various signaling pathways that support proliferation, cellular growth and the survival of MM cells [171, 172]. Proteasomal inhibition induces multiple apoptotic pathways that include the induction of endoplasmic reticulum stress response [173, 174]. Proteasomal inhibition also inhibits NF-κB signaling, there by downregulating cell adhesion molecules, angiogenesis factors and cytokine signaling in the microenvironment. Immunomodulation shows promise in delaying the progression from asymptomatic (smoldering) to symptomatic (active) MM [172].

1.6 Chronic myeloid leukemia (CML)

CML is characterized by the reciprocal translocation involving the long arms of chromosome 9 and 22, t(9;22), the Philadelphia chromosome [175, 176]. This translocation, juxtaposition of the 3' sequence from ABL proto-oncogene on chromosome 9, with the 5' sequence from BCR (break point cluster region) gene on chromosome 22, results in the fusion oncogene, BCR-ABL. CML is characterized by the over production of hematopoietic stem cells carrying the BCR-ABL translocation in the peripheral blood in the chronic phase (CP). With the progression of the disease, the patient enters an acute phase (AP) followed by the blast phase (BP), during which the differentiation of hematopoietic cells is arrested with the accumulation of immature blasts in the bone marrow and peripheral blood [177].

The ABL gene encodes for a non-receptor tyrosine kinase carrying SRC homology domains (SH1-SH3) at the amino terminal. The SH1 domain is responsible of the kinase activity while the SH2 and SH3 domains facilitates protein-protein interactions [178]. The ABL protein is nuclear localized with a nuclear localizing signal, DNA binding and actin binding motifs at the carboxylic terminal [179]. With the BCR-ABL chromosomal translocation in CML cells, the resulting fusion protein, a deregulated thyrosine kinase is localized in the cytoplasm allowing access to many cytoplasmic substrates. As a consequence of the fusion protein, a number of signaling pathways that sustain cellular proliferation are activated (RAS, MAPK, JAK-STAT, PI3K, Myc). STAT5 plays an important role in many biological processes that includes lymphocyte development and its function [180]. The deregulated kinase activity also inhibits the apoptotic pathways induced by DNA damage or by the withdrawal of growth factors [181]. Thus, CML patients have a clonal expansion of hematopoietic cells that express the BCR-ABL fusion protein, thus making the tyrosine kinase an obvious therapeutic target for controlling the disease.

The very first therapy was hydroxyurea or busulfan which had little or negligible effect on the disease outcome. Later studies demonstrated that INFα had a positive effect in the management of CML, proving to be promising when compared to hydroxyurea and bisulfan [182, 183]. With the better understanding of the mechanism of the constitutive kinase activity of BCR-ABL in the pathogenesis of CML, molecular targeting of the kinase by chemical

inhibitors was initiated. Imatinib mesylate (IM), a tyrosine kinase inhibitor has been the most effective treatment so far of Ph positive CML [184]. Despite the positive clinical outcome of IM treatment, there are a considerable number of CML patients developing resistance to the drug, leading to the development of next generation tyrosine kinase inhibitors such as nilotinib (NI) and dasatinib. The resistance to IM and NI are poorly understood. They have been attributed to point mutations and other BRC-ABL independent mechanisms like the role of the microenvironment. A couple of studies indicate that INFγ levels are elevated in patients treated with IM [185]. Also, treatment with IM does not eradicate CML cells entirely, CML stem cells remaining unaffected [186]. These stem cells, despite their small number are capable of reinitiating leukemia upon discontinuation of tyrosine kinase inhibitor (IM) treatment.

1.7 Interferon gamma (INFγ)

The discovery of interferons (INF) was initially associated with biological activity during viral infections [187]. Subsequent studies demonstrated its diverse effects on cellular functions including antimicrobial responses, apoptosis and control of cell cycle [188-191]. INFs are secreted soluble protein molecules classified based on their receptor binding specificity and sequence homology. Type I INFs (INFα, β, ω and τ) share structural similarity and bind to a common heterodimeric receptor - INFα/β receptor. Most cell types secrete traces of type-I INFs, however INFα and ω are predominantly secreted by hematopoietic cells and INFβ by fibroblasts and activated macrophages [192, 193]. INFγ is the only type II IFN that is secreted predominantly by activated T and NK cells, but less often by APCs, B and NKT cells [194-196]. Cytokines IL12 and IL18 secreted by APCs promote INFγ production by stimulating T, NK cells and macrophages during immune response to infections.

Signaling of INFγ from the cell surface is transduced through the Janus family of kinase (JAKs: JAK1-3 and Tyk2) - signal transducer and activator of transcription (STATs1-6, including STAT5a and STAT5b) to the nucleus of the cell. This signal transduction machinery involves sequential receptor recruitment and the activation of JAKs and the STATs. The heterodimerisation of two molecules each of INFGR1 and 2 constitute the INFγ receptor [197]. The binding of biologically active INFγ dimer to IFNGR1 triggers the autophosphorylation and activation of JAK2 and the subsequent JAK1 phosphorylation by JAK2 [198]. An activated JAK1 molecule phosphorylates the two IFNGR1 chains at tyrosine 440, creating docking sites for the SH2 domain containing STAT1 molecules [199]. The recruitment of STAT1 molecules to the INFGR1 results in the JAK2 mediated canonical phosphorylation of STAT1 at the C terminal tyrosine 701 [200]. The four critical phosphorylation steps until this stage (in sequence, JAK2-JAK1-INFGR1-STAT1) occur within as short as one minute of the cells exposed to INFγ. Phosphorylation of STAT1 results in its homodimerization and dissociation form IFNGR1 [199]. Dissociated STAT1 dimers enter the nucleus to regulate transcription of specific target genes via binding to specific response elements on DNA [201, 202]. The transcription initiation of INFγ induced genes

occurs within 30 minutes of INFγ stimulation of cells, transcriptional factor like IRF-1 being among the first to be transcribed.

1.7.1 Regulation of STAT activity

The STAT proteins consist of seven conserved domains. The amino terminal is implicated in the homotypic dimerization of inactive STAT molecules. This domain is known to facilitate STAT binding to tandem γIFN activation site (GAS) elements on the DNA and it also plays a role in the cytoplasmic-nuclear shuttle of STAT molecules [203, 204].

The characteristic feature of STAT signaling is its rapid turnover, with a sharp initiation of signaling and subsequent decay. Activated STAT molecules rapidly accumulate in the nucleus of the cell, but following the signal decay the STAT molecules are exported back to the cytoplasm to carry out the next wave of signaling. The signal decay is strongly associated with downregulation of cytokine receptors, JAK and also the STAT's transcriptional function. The activation of STAT molecules holds the key for the cytokine elicited signal transduction to reach the nucleus and transactivate specific target genes. Thus mechanisms that decay STAT signaling are critical in the rapid turnover of STAT signaling. Three mechanisms that are involved in the decay of STAT signaling are well characterized:

Dephosphorylation: Phosphatases play a crucial role in regulating the activity of kinase based STAT signaling. Phosphatases like SHP-1, 2 and CD45 have been associated with the decay of cytokine mediated Jack-STAT signaling [205, 206]. Later studies identified the role of PTP1B, TC-PTP and PTP-BL in dephosphorylation of STATS [206, 207]. Among these phosphatases, SHP-2 and TC-PTP have been implicated in the dephosphorylation of STAT molecules in the nucleus, a critical step in STAT signal decay and its nuclear export [204].

Cytoplasmic-Nuclear shuttle: The nuclear accumulation of STAT is an indication of the active state of the molecule. The predominant site of localization during inactive state is the cytoplasm. The nuclear import and export of STAT molecules is tightly regulated by nuclear export sequence (NES) and nuclear localization sequence (NLS) elements [208].

Regulation by SOCS: STAT activation is directly antagonized by its own set of transcriptional target - suppressors of cytokine signaling (SOCS) proteins (SCOS1-7 and CIS), resulting in a feedback loop regulation. STAT molecules directly regulate the expression of SOCS1, SOCS3 and CIS but not SOCS2 [209], for example in mouse fibroblast cell line NIH-3T3, INFγ induces SOCS1 and SOCS3 expression [210, 211]. The STAT induced SOCS proteins negatively modulate the JAK-STAT signaling by competing with the STAT molecules to bind to the phosphorylated tyrosine sites on the receptor and also by binding to JAK molecules thorough their SH2 domain and inhibiting their activity. The SH2 domains on the SOCS proteins function like an adaptor and thus can facilitate the ubiquitination and subsequent proteosomal degradation of associated signaling proteins [212].

1.7.2 Non-canonical modifications of STAT

Serine phosphorylation: with the exception of STAT2 all other STATS are phosphorylated on the serine residues on the transcriptional activation domain (TAD) [213]. Phosphorylation occurs on conserved serine residues situated in PMS*P motif (Ser727 in STAT1 and 3, Ser721in STAT4), PS*P motif (Ser725 in STAT5a and Ser730 in STAT5b) and SS*PD motif (Ser756 in STAT6) [214]. STAT1 and 5 have an additional phosphorylation site, Ser708 and Ser779 respectively. *In vitro* studies have identified the following kinases involved in STAT modifications: MAPK (p38MAPK: STATs1,3,4; ERK: STAT3,5; JNK: STAT3), PKCδ (STAT1 and 3), mTOR (STAT3), NLK (STAT3) and CAMKII and IIKε (STAT1) [213, 215]. The phosphorylation of serine regulates the transcriptional activity of STATs. A mouse model with a STAT1S727A mutation has defective INFγ mediated innate immune response [216]. Also, T cells carrying STAT4 S721A mutation show impaired IL12 following INFγ stimulation [217].

Acetylation: Reversible acetylation of lysine residues on STAT1, 3 and 6 have been reported to regulate their function. Acetylation of STAT1 and 3 impairs NFκB signaling, resulting in a STAT1 mediated pro-apoptotic and STAT3 mediated anti-apoptotic effect [217, 218]. Acetylation of STAT3 contributes to the regulation of its transcriptional function and to the stability of its homodimers [219, 220].

Glycosylation: O-glycosylation of Thr92 on STAT5 increases its affinity to the co-activator CBP, thus increasing the transcriptional function of STAT5. The Thr92 residue is conserved in STAT1, 3 and 6 [221].

1.8 BCL6

BCL6 is a transcriptional factor characterized by an amino terminal BTB/POZ domain and six zinc finger DNA binding motifs at the carboxylic terminal [222]. The intermediate backbone constitutes of three PEST domains involved in protein stabilization and activity [223]. BCL6 is an oncogenic master gene regulator, which may suppress the expression of over 1200 genes. The target genes are repressed through direct recruitment of class I and II histone deacetylase complex (HDAC) or via the recruitment of other co-repressors [224, 225]. Depending on the recruitment of a specific co-repressor, different subsets of target genes are repressed. Most often transcriptional activity of BCL6 is accomplished by its binding to specific DNA motifs via the carboxylic terminal zinc finger domain. Interferon regulatory factor 4 (IRF4) and STATs have been established to compete with BCL6 for certain promoters of target genes. Transcriptional factors FoxO1, FoxO3a, FoxO4, STAT1, STAT3, and IRF8 are shown to induce BCL6 expression [226, 227].

The most significant role of BCL6 is in the regulation of transcriptional program preventing premature activation and differentiation of germinal center (GC) B cells [228, 229]. Somatic hypermutations (SHM) and class switch recombination (CSR) in B cells during the GC reaction increases immunoglobulin diversity and affinity for antigens. The execution of SHM and CSR involves double stranded DNA brakes, a damage which should trigger a p53

response [230]. However, CG B cells tolerate these dsDNA breaks, though BCL6 mediated suppression of p53 [231].

BCL6 auto regulates its transcription by binding to the 5' promoter region and suppressing its own transcription. This is substantiated by the identification of mutations in the BCL6 binding sites on the first noncoding exon of the gene that prevents BCL6 binding, thus impending its negative regulation and potentially contributing to lymphoma genesis [232]. BCL6 promoter has two regions (region A and B), each with several STAT binding motifs [233]. ChIP sequencing data set from various human cell types show STAT3 binding to both regions while STAT5 binds to only region B. STAT5 and STAT3 have been shown to reciprocally regulate BCL6 expression in breast cancer cell lines, STAT3 induces while STAT5 repressed BCL6 expression [234]. This repression by STAT5 is dominant over STAT3 mediated induction.

During B cell differentiation towards memory and plasma cells, post transcriptional modifications of BCL6 in the PEST domain also regulates its function. Acetylation of lysine residues by p300 in the PEST domain of BCL6 has been associated with impaired recruitment of co-repressors to target site [235]. Antigen engagement of BCR activates the MAPK pathway, phosphorylation of serine residues my MAPK following BCR engagement allows ubiquitilation and the subsequent proteasomal degradation. CD40-CD40L signaling in the GC B cells suppresses BCL6 transcription by the binding of NF-κB induced IRF4 to the promoter region of BCL6 [236].

The oncogenic role of BCL6 was first established in DLBCL, an outcome of the chromosomal translocation 3q27 [237]. Heterologous promoter juxtaposed to the 5'-end of BCL6 gene drives its expression. Promoters of genes that are constitutively active in B cell lineage such as, IgH, IgL, PAX5 and H4 often drive BCL6 expression in tumors originating from the GC [238]. Given the fact that BCL6 provides tolerance to dsDNA breaks in GC B cells, it may prove a persistent tolerance to DNA damage, thus abetting the accumulation of oncogenic mutations [239]. The wide spread expression of BCL6 in GC derived lymphomas make it a potential therapeutic target.

2 AIM OF THIS THESIS

The following questions were addressed in the thesis,

1. Does p53 contribute to the regulation of homeostasis in activated T cells?

2. Are CD4+ T cells involved in the modulation of EBV gene expression?

3. Does INFγ regulate BCL6 expression in MM and IM treated CML cells?

3 RESULTS AND DISCUSSION

p53 contributes to the regulation of T cell homeostasis through the induction of pro-apoptotic SAP and suppression of cMyc in activated T cells (Papers I and II)

Results from our laboratory have previously shown that wt p53 induces SAP expression in Burkitt lymphoma cell lines following DNA damage [240] and led to the discovery of SAP's pro-apoptotic role in T cells [241, 242] and the induction of p53 in activated T cells [241], suggesting its involvement in T cell homeostasis.

As a next step we studied the kinetics of p53, SAP, cMyc, p14ARF and p21 expression in activated T cells. We showed that p53 and pro-apoptotic SAP expression was induced in activated but not in un-stimulated T cells. The kinetics of SAP expression level inversely corresponded to the proliferation of activated T cells. Proliferation of T cells during the initial activation reached a maximum and expressed lower levels of SAP, but in cells that showed decreased proliferation, SAP expression was elevated. The kinetics of SAP expression corresponded to the levels of cleaved PARP, also indicating that activated T cells expressing higher levels of SAP undergo apoptosis.

The E3 ubiquitin ligase-MDM2 inhibitor, nutlin-3, facilitated selective induction of p53 [243] in un-stimulated T cells accompanied by SAP mRNA and protein induction, indicating that p53 induces SAP expression in T cells. Also, chromatin immune-precipitation assay confirmed the binding of p53 to SAP promoter region in activated T cells. Thus, we demonstrate that SAP is a transcriptional target of p53 in activated primary T cells and hence underlined the involvement of p53 in the regulation of T cell homeostasis through pro-apoptotic SAP.

cMyc was induced in activated T cells and the kinetics of its expression corresponded to the proliferation of activated T cells. cMyc target p14ARF accompanied the induction of p53 and p21 in activated T cells. Expression p14ARF and p53 remained high thought, while p21 expression was induced only during the initial activation and declined with time. Various studies have well established that p14ARF binding to MDM2 stabilizes p53 protein. This binding inhibits p53 degradation which otherwise is mediated through the formation of the MDM2-p53 complex and ubiquitination of p53 by MDM2 for proteosomal degradation. Thus cMyc can positively regulate p53 expression through p14ARF. At the other end, it is also established that p53 can suppress cMyc transcription. This motivated us to explore the involvement of cMyc-p53 network in regulating cell growth and survival in a model where cMyc and p53 pathways were unaltered. To this end, we selectively induced p53 with MDM2 inhibitor nultin-3 and inhibited cMyc with small molecule 10058-F4 [244]. Nultin-3 treatment of activated T cells further potentiated p53 along with its transcriptional target p21, but decreased cMyc expression and inhibited proliferation. Downregulation of cMyc accompanied low levels of p14ARF expression. Also, treatment of activated T cells with

nutlin-3 for a short duration (8 h) showed decreased cMyc mRNA, while p53 inducible p21 mRNA was unregulated, indicating that elevated levels of p53 suppresses cMyc transcription.

As activation of notch signaling during T cell immune response induces cMyc that drives the expansion of T cell clones, we tested the consequence of DAPT mediated Notch inhibition [245] on the expression of the above proteins and cell proliferation. Notch inhibition in activated T cells downregulated cMyc expression and decreased proliferation, correspondingly p14ARF and p53 expression were also downregulated. This suggested that p53 induced in activated T cells follow the cMyc-p14ARF pathway. cMyc was specifically inhibited using 10058-F4 to further confirm the involvement of cMyc-p14ARF in the induction of p53 in activated T cells. 10058-F4 treatment of activated T cells decreased cell proliferation, cMyc expression and also lowered p14ARF and p53 expression. This confirmed the involvement of cMyc-p14ARF in the induction of p53 in activated T cells. Nulin-3 induced p53 and 10058-F4 mediated cMyc inhibition induced both apoptosis and growth arrest in activated T cells. The cytotoxic function of activated T cells was retained despite the inhibitory effect of nutlin-3 and cMyc inhibitor 10058-F4 on proliferation.

Thus, we establish the contribution of p53 in T cell homeostasis through its regulation of pro-apoptotic SAP and proto-oncogene cMyc.

EBV gene expression modulated by CD4+ secreted soluble factors (Paper III)

EBV infects naïve IgD+ B cells, subsequently expressing nine viral encoded proteins (latency III), transforming cells into immunoblasts. CD8+ T cell response eliminates the majority of type III cells, while a fraction of infected cells migrate to the lymph node follicles where it undergoes changes to express only three viral proteins – EBNA1, LMP1 and LMP2 (latency II). Previous results from our group have studied the changes in EBV latency pattern induced through cytokines secreted by CD4+ T cells. IL4, IL10 and IL13 induced the shift from type I BL and EBV positive HL cell lines to type IIa latency, while IL21 induced a switch from latency III to IIa. Based on these findings we studied the plausible role of activated CD4+ T cells in altering EBV gene expression pattern.

Activated CD4+ T cells co-cultured with autologous or allogenic LCLs downregulated EBNA2 expression, indicating a shift from latency III towards latency IIa. Downregulation of EBNA2 was accompanied by the concomitant decrease in proliferation. The co-culture system does not corroborate whether EBNA2 downregulation was CD4+ T cell contact dependent or not. In order to address this, the transwell (TW) experimental system was exploited, where the LCLs were confined to the insert and the activated CD4+ T cell were cultured at the bottom of the well. Similar to the co-culture, activated CD4+ T cells in the TW system downregulated EBNA2 expression, but upregulated LMP1. This phenomenon was not observed with CD8+ T cells. Decrease in EBNA2 was accompanied by the decrease in CD23, a B cell activation marker upregulated in cells with latency III. The changes in

EBNA2 and LMP1 expression were also confirmed at the transcriptional level. The decrease in EBNA2 mRNA transcripts correlated with decreased Cp activity, suggesting a switch from latency III viral program. However, Qp activity remained unchanged, indicating only a partial switch to latency IIa.

Based on our previous knowledge that IL21 negatively regulates EBNA2 and positively regulates LMP1 [246], neutralizing anti IL21 was added to the TW system. Functional inhibition of IL21 in TW experiments decreased LMP1 expression, but did not restore expression of EBNA2 to its initial level. This suggested that IL21 was only accountable for the upregulation of LMP1, and other soluble factor/s were involved in the regulation of EBNA2.

Based on an earlier finding that CD40L can downregulate EBNA2 expression in LCLs [247], neutralizing anti-CD40 antibody was introduced in the TW experiments. Partial inhibition of EBNA2 expression was achieved with this treatment. Thus, we demonstrate the role of soluble factors secreted by CD4+ T cells in regulating EBV latency switch from type III to II.

Interferon γ induces BCL6 expression in multiple myeloma and in imatinib treated chronic myeloid leukemia cells through STAT1 (Paper IV)

BCL6 is a transcriptional factor essential for the normal development of T and B lymphocytes. Deregulation of BCL6 expression is strongly associated with the pathogenesis of several hematological malignancies such as, DLBCL, BL and MM. Also, BCL6 expression is essential for the survival of Ph positive CML stem cells resisting IM treatment. It has been reported that T cells in IM treated CML patients secrete increased levels of INFγ [185]. In addition, INFγ has been reported to modulate the response of CML cells to TKIs treatment. Based on earlier reports of BCL6 induction through INFγ signaling in T cells and keratinocytes [248-250], we investigated the plausibility of INFγ induced BCL6 expression in MM and CML cells.

To this end, BCL6 expression was tested in MM, CML and IM treated CML cells following the treatment with several well characterized growth/survival factors. Rapid and strong induction of BCL6 mRNA was observed in MM and IM treated CML cell treated with INFγ. Both variants of BCL6 mRNA were induced upon INFγ treatment, suggesting that both regions A and B on the BCL6 promoter are activated. Induction of BCL6 was independent of *de novo* protein synthesis.

INFγ activates several pathways besides the classical JAK/STAT1 signaling pathway. However, only STAT1 was phosphorylated following INFγ treatment in untreated and IM treated CML cells. IM inhibited BCR-ABL mediated constitutive phosphorylation of STAT5 in CML cells, untreated or treated with INFγ. From our observations in CML cells, INFγ induced BCL6 expression was restricted only to cells treated with IM, which rendered STAT5 inactive. It has been shown that STAT5 displays a dual effect on gene regulation,

inducing or repressing transcription of target genes. STAT5 homodimer binds to specific DNA motifs initiating transcription, whereas STAT5 tetrameric complex can also bind to STAT5 specific binding sites, but in this instance repress transcription [251]. Tetrameric STAT5 complexes interact with histone H3 Lys27 (H3K27) methylase Ezh2, forming a H3K27-trimethylated repressive chromatin [252]. This suggested that the constitutive phosphorylated STAT5 might have an inhibitory effect on BCL6 transcription in CML cells not treated with IM. Specific inhibition of STAT5 in CML cells treated with INFγ alone induced strong BCL6, without affecting transcription of IRF1. This also suggested the dominant repressive role of pSTAT5 regulation of BCL6 in CML cells treated with INFγ. As a next step, Ph positive stem cells were isolated from peripheral blood of untreated CML patients to examine BCL6 expression following INFγ and IM treatment. BCL6 transcription was induced only in CML stem cells treated with combination of INFγ and IM. However transcription of IRF1 increased with INFγ treatment, not affected by the presence or absence of IM.

Similarly to the effect of INFγ on CML cells, STAT1 was phosphorylated, in INFγ treated MM cells. However, INFα treatment of these cells induced a much stronger STAT1 phosphorylation than INFγ, but also very strong STAT5 phosphorylation accompanied by the lack in BCL6 expression. Based on the earlier finding of the dominant repressive inhibition effect of STAT5 over STAT1 in CML cells, downregulation of STAT5 by siRNA in MM cells treated with INFα induced strong BCL6 transcription. Expression of ISG56 was unaltered with the downregulation of STAT5. This confirmed STAT5's dominant repressive effect on BCL6 expression also in MM cells.

Our finding suggests that INFγ through STAT1 induces BCL6, an oncogenic master regulator critical for the survival of MM and IM treated CML stem cells.

4 ACKNOWLEDGEMENTS

People around me make my world. It is always easier and fun to complete a long journey with such supportive people. I thank you all for being with me.

Though I am a Ph.D. student at the Department of Oncology-Pathology, KI, all my work has been carried out at the Department of Microbiology, Tumor and Cell Biology, KI.

It all began with a mail from me to Eva Klein enquiring about the possibility of joining her group as a PhD student. Very soon I received a reply from George Klein showing their willingness to accommodate me in the group. Dear Eva and George, thank you for the opportunity you provided. It has been a fantastic learning experience and a privilege to be a part of your group.

Eva, thank you for your keen interest in my work, for the many discussions, for sharing innumerable number of stories and everything else. You are a bundle of energy, which is infectious.

George, thank you for sharing your insights into tumor biology and beyond. I find you having 'Zen' like calmness in your thoughts.

Klas Wiman, thank you for accepting to be my main supervisor, for your interest in my project and my progress as a researcher.

Anjali Karande, my supervisor at the Dept. of Biochemistry, I.I.Sc., Bangalore. Thank you for the opportunity you gave me to be a part of your group, for introducing me to research and for creating a wonderful environment to learn and explore.

Present and past members of the Klein's group: Barbro, Benedek, Benjamin (Ben), Daniel (Dani), Dorina, Eahsan, Elena-Vladimir, Hyrettin, Liang, Lorand (Lori), Maria (Mia), Monika, Mushtaq, Noémi, Sri Harsha, Suhas, Tatiana (Tanya), Tomek, Twana and thesis students, Christina and Marijke. Thanks to Tanya and Twana for their enthusiasm to design the thesis cover.

Noémi, thank you for your support throughout, for mentoring me with laboratory techniques and writing skills. To me, EBV would be a dragon, but for you.

Dani, thank you for sharing your expertise in molecular biology techniques, for introducing me to BCL6.

Twana, Mushtaq and Benedek, thank you for the coffee time, lunchtime, for sharing reagents, for the scientific and political discussions and for sharing your memories from back home.

Barbro and Mia - the wonder women. Thank you for the amazing and relentless co-ordination that keeps every little and big thing rolling in clockwork precession and for sharing stories from the ‘old tumor biology department.’

Sonia, thank you for your support and interest in my work. Discussions with you are always exciting.

Ingemar and group members, Li-fu, Jie-Zhi, Liudmila and Quin for the ever interesting joint group meetings. Ingemar, thank you for organizing tumor biology lectures.

Friends from CCK, Ema, Lidi and Qiang, thank you for sharing reagents and inviting me for Christmas dinners.

Present and past colleagues in MTC C4 corridor, Bence, Francesca, Katarina, Magdalena, Pontus, Sylvie, Vishal, thank you for sharing equipment, reagents and organizing the corridor Christmas glögg.

Martin, thank you for your kind words.

Friends from Jagagatan, Diana, Lea, and Nadim, thank you for your friendship.

Past and present members of MTC, Chaniya, Mariam and Sadia,

Thank you Rom for being the engine powering our badminton group, Chaniya, we miss you. Thanks to my Indian friends at KI, Aditya, Ankit-Ishani, Anuj, Ashwini-Suhas, Bhavya-Srikanth, Deepti-Lalit, Himjoth, Jagadeesh, Pradeep, Vishnu Priya-Akilan, Shahul, Shawon, Sridhar, Suman, Sunitha-Sakthi, Srimanta, Ranjana and Reddyji.

Special thanks to Carina, Chaniya, Lalit, Sridhar, Suhas and Suman for all the great time spent together. Carina-Damian and Shawon-Sajit, thank you for the amazing trip to Kiruna.

Divya-Naveen, Lakshmi-Nikhil, Praghna-Swaroop, Shruthi-Harisha, Sonam-Samarth, Vinutha-Jayanth, Chethan, Srikanth, Vinay and Vikram, thank you for everything, you are my family in Stockholm.

Thank you Ashish bhai, Vishal bhai and Krushna for all the quality time and cooking experiments. Thank you also for sharing your insights into public health.

My beloved family - my parents, Indira and Sekharappa, my brother, Vishnu, thanks to for all the love, support and encouragement. I have immensely missed being with you all these years. My wife, Shreya, you are wonderful, I fall short of words to describe what I feel, I can’t thank you enough, I can only love you.

The study was supported by grants from the Swedish Cancer Society (*Cancerfonden*) and the Cancer Research Institute (New York)/Concern Foundation (Los Angeles).

5 REFERENCES

1. Klein G and Klein E. Immune surveillance against virus-induced tumors and nonrejectability of spontaneous tumors: contrasting consequences of host versus tumor evolution. Proc Natl Acad Sci U S A. 1977; 74(5):2121-2125.
2. Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144(5):646-674.
3. Taub R, Kirsch I, Morton C, Lenoir G, Swan D, Tronick S, Aaronson S and Leder P. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proc Natl Acad Sci U S A. 1982; 79(24):7837-7841.
4. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, Leiserson MD, Miller CA, Welch JS, Walter MJ, Wendl MC, Ley TJ, et al. Mutational landscape and significance across 12 major cancer types. Nature. 2013; 502(7471):333-339.
5. Lane DP and Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. Nature. 1979; 278(5701):261-263.
6. Linzer DI and Levine AJ. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell. 1979; 17(1):43-52.
7. Finlay CA, Hinds PW and Levine AJ. The p53 proto-oncogene can act as a suppressor of transformation. Cell. 1989; 57(7):1083-1093.
8. Lechner MS and Laimins LA. Inhibition of p53 DNA binding by human papillomavirus E6 proteins. J Virol. 1994; 68(7):4262-4273.
9. Pise-Masison CA, Choi KS, Radonovich M, Dittmer J, Kim SJ and Brady JN. Inhibition of p53 transactivation function by the human T-cell lymphotropic virus type 1 Tax protein. J Virol. 1998; 72(2):1165-1170.
10. Kashuba E, Yurchenko M, Szirak K, Stahl J, Klein G and Szekely L. Epstein-Barr virus-encoded EBNA-5 binds to Epstein-Barr virus-induced Fte1/S3a protein. Exp Cell Res. 2005; 303(1):47-55.
11. Yi F, Saha A, Murakami M, Kumar P, Knight JS, Cai Q, Choudhuri T and Robertson ES. Epstein-Barr virus nuclear antigen 3C targets p53 and modulates its transcriptional and apoptotic activities. Virology. 2009; 388(2):236-247.
12. Zhang Q, Gutsch D and Kenney S. Functional and physical interaction between p53 and BZLF1: implications for Epstein-Barr virus latency. Mol Cell Biol. 1994; 14(3):1929-1938.
13. Mowat M, Cheng A, Kimura N, Bernstein A and Benchimol S. Rearrangements of the cellular p53 gene in erythroleukaemic cells transformed by Friend virus. Nature. 1985; 314(6012):633-636.
14. Ben David Y, Prideaux VR, Chow V, Benchimol S and Bernstein A. Inactivation of the p53 oncogene by internal deletion or retroviral integration in erythroleukemic cell lines induced by Friend leukemia virus. Oncogene. 1988; 3(2):179-185.

15. Wu L and Levine AJ. Differential regulation of the p21/WAF-1 and mdm2 genes after high-dose UV irradiation: p53-dependent and p53-independent regulation of the mdm2 gene. Mol Med. 1997; 3(7):441-451.
16. Rodriguez MS, Desterro JM, Lain S, Lane DP and Hay RT. Multiple C-terminal lysine residues target p53 for ubiquitin-proteasome-mediated degradation. Mol Cell Biol. 2000; 20(22):8458-8467.
17. Leng RP, Lin Y, Ma W, Wu H, Lemmers B, Chung S, Parant JM, Lozano G, Hakem R and Benchimol S. Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. Cell. 2003; 112(6):779-791.
18. Dornan D, Wertz I, Shimizu H, Arnott D, Frantz GD, Dowd P, O'Rourke K, Koeppen H and Dixit VM. The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature. 2004; 429(6987):86-92.
19. Chen D, Kon N, Li M, Zhang W, Qin J and Gu W. ARF-BP1/Mule is a critical mediator of the ARF tumor suppressor. Cell. 2005; 121(7):1071-1083.
20. Shieh SY, Ikeda M, Taya Y and Prives C. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. Cell. 1997; 91(3):325-334.
21. Saito S, Goodarzi AA, Higashimoto Y, Noda Y, Lees-Miller SP, Appella E and Anderson CW. ATM mediates phosphorylation at multiple p53 sites, including Ser(46), in response to ionizing radiation. J Biol Chem. 2002; 277(15):12491-12494.
22. Shieh SY, Ahn J, Tamai K, Taya Y and Prives C. The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. Genes Dev. 2000; 14(3):289-300.
23. Pabla N, Huang S, Mi QS, Daniel R and Dong Z. ATR-Chk2 signaling in p53 activation and DNA damage response during cisplatin-induced apoptosis. J Biol Chem. 2008; 283(10):6572-6583.
24. Yang S, Kuo C, Bisi JE and Kim MK. PML-dependent apoptosis after DNA damage is regulated by the checkpoint kinase hCds1/Chk2. Nat Cell Biol. 2002; 4(11):865-870.
25. Tang Y, Zhao W, Chen Y, Zhao Y and Gu W. Acetylation is indispensable for p53 activation. Cell. 2008; 133(4):612-626.
26. Liu L, Scolnick DM, Trievel RC, Zhang HB, Marmorstein R, Halazonetis TD and Berger SL. p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage. Mol Cell Biol. 1999; 19(2):1202-1209.
27. Chuikov S, Kurash JK, Wilson JR, Xiao B, Justin N, Ivanov GS, McKinney K, Tempst P, Prives C, Gamblin SJ, Barlev NA and Reinberg D. Regulation of p53 activity through lysine methylation. Nature. 2004; 432(7015):353-360.
28. Kahyo T, Nishida T and Yasuda H. Involvement of PIAS1 in the sumoylation of tumor suppressor p53. Mol Cell. 2001; 8(3):713-718.
29. Liu X, Wang D, Zhao Y, Tu B, Zheng Z, Wang L, Wang H, Gu W, Roeder RG and Zhu WG. Methyltransferase Set7/9 regulates p53 activity by interacting with Sirtuin 1 (SIRT1). Proc Natl Acad Sci U S A. 2011; 108(5):1925-1930.
30. el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW and Vogelstein B. WAF1, a potential mediator of p53 tumor suppression. Cell. 1993; 75(4):817-825.
31. el-Deiry WS, Harper JW, O'Connor PM, Velculescu VE, Canman CE, Jackman J, Pietenpol JA, Burrell M, Hill DE, Wang Y and et al. WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. Cancer Res. 1994; 54(5):1169-1174.

32. Brugarolas J, Moberg K, Boyd SD, Taya Y, Jacks T and Lees JA. Inhibition of cyclin-dependent kinase 2 by p21 is necessary for retinoblastoma protein-mediated G1 arrest after gamma-irradiation. Proc Natl Acad Sci U S A. 1999; 96(3):1002-1007.
33. Satyanarayana A, Hilton MB and Kaldis P. p21 Inhibits Cdk1 in the absence of Cdk2 to maintain the G1/S phase DNA damage checkpoint. Mol Biol Cell. 2008; 19(1):65-77.
34. Hermeking H, Lengauer C, Polyak K, He TC, Zhang L, Thiagalingam S, Kinzler KW and Vogelstein B. 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. Mol Cell. 1997; 1(1):3-11.
35. Chan TA, Hermeking H, Lengauer C, Kinzler KW and Vogelstein B. 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage. Nature. 1999; 401(6753):616-620.
36. Puzio-Kuter AM. The Role of p53 in Metabolic Regulation. Genes Cancer. 2011; 2(4):385-391.
37. Horvitz HR. Genetic control of programmed cell death in the nematode Caenorhabditis elegans. Cancer Res. 1999; 59(7 Suppl):1701s-1706s.
38. Renehan AG, Booth C and Potten CS. What is apoptosis, and why is it important? BMJ. 2001; 322(7301):1536-1538.
39. Budihardjo I, Oliver H, Lutter M, Luo X and Wang X. Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol. 1999; 15:269-290.
40. Suliman A, Lam A, Datta R and Srivastava RK. Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. Oncogene. 2001; 20(17):2122-2133.
41. Ashkenazi A and Dixit VM. Death receptors: signaling and modulation. Science. 1998; 281(5381):1305-1308.
42. Dhein J, Walczak H, Baumler C, Debatin KM and Krammer PH. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). Nature. 1995; 373(6513):438-441.
43. Chicheportiche Y, Bourdon PR, Xu H, Hsu YM, Scott H, Hession C, Garcia I and Browning JL. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J Biol Chem. 1997; 272(51):32401-32410.
44. Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X and Akey CW. Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. Mol Cell. 2002; 9(2):423-432.
45. Deveraux QL and Reed JC. IAP family proteins--suppressors of apoptosis. Genes Dev. 1999; 13(3):239-252.
46. Hegde R, Srinivasula SM, Zhang Z, Wassell R, Mukattash R, Cilenti L, DuBois G, Lazebnik Y, Zervos AS, Fernandes-Alnemri T and Alnemri ES. Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction. J Biol Chem. 2002; 277(1):432-438.
47. Du C, Fang M, Li Y, Li L and Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell. 2000; 102(1):33-42.
48. Sakahira H, Enari M and Nagata S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature. 1998; 391(6662):96-99.
49. Gross A, McDonnell JM and Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. Genes Dev. 1999; 13(15):1899-1911.

50. Oltvai ZN, Milliman CL and Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell. 1993; 74(4):609-619.
51. Yin XM, Oltvai ZN, Veis-Novack DJ, Linette GP and Korsmeyer SJ. Bcl-2 gene family and the regulation of programmed cell death. Cold Spring Harb Symp Quant Biol. 1994; 59:387-393.
52. Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P and Moll UM. p53 has a direct apoptogenic role at the mitochondria. Mol Cell. 2003; 11(3):577-590.
53. Slee EA, Adrain C and Martin SJ. Executioner caspase-3, -6, and -7 perform distinct, non-redundant roles during the demolition phase of apoptosis. J Biol Chem. 2001; 276(10):7320-7326.
54. Fischer U, Janicke RU and Schulze-Osthoff K. Many cuts to ruin: a comprehensive update of caspase substrates. Cell Death Differ. 2003; 10(1):76-100.
55. Li T, Kon N, Jiang L, Tan M, Ludwig T, Zhao Y, Baer R and Gu W. Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. Cell. 2012; 149(6):1269-1283.
56. Valente LJ, Gray DH, Michalak EM, Pinon-Hofbauer J, Egle A, Scott CL, Janic A and Strasser A. p53 efficiently suppresses tumor development in the complete absence of its cell-cycle inhibitory and proapoptotic effectors p21, Puma, and Noxa. Cell Rep. 2013; 3(5):1339-1345.
57. Hellborg F, Qian W, Mendez-Vidal C, Asker C, Kost-Alimova M, Wilhelm M, Imreh S and Wiman KG. Human wig-1, a p53 target gene that encodes a growth inhibitory zinc finger protein. Oncogene. 2001; 20(39):5466-5474.
58. Mendez-Vidal C, Wilhelm MT, Hellborg F, Qian W and Wiman KG. The p53-induced mouse zinc finger protein wig-1 binds double-stranded RNA with high affinity. Nucleic Acids Res. 2002; 30(9):1991-1996.
59. Vilborg A, Glahder JA, Wilhelm MT, Bersani C, Corcoran M, Mahmoudi S, Rosenstierne M, Grander D, Farnebo M, Norrild B and Wiman KG. The p53 target Wig-1 regulates p53 mRNA stability through an AU-rich element. Proc Natl Acad Sci U S A. 2009; 106(37):15756-15761.
60. Mazan-Mamczarz K, Galban S, Lopez de Silanes I, Martindale JL, Atasoy U, Keene JD and Gorospe M. RNA-binding protein HuR enhances p53 translation in response to ultraviolet light irradiation. Proc Natl Acad Sci U S A. 2003; 100(14):8354-8359.
61. Takagi M, Absalon MJ, McLure KG and Kastan MB. Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. Cell. 2005; 123(1):49-63.
62. Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, Feldmann G, Yamakuchi M, Ferlito M, Lowenstein CJ, Arking DE, Beer MA, Maitra A and Mendell JT. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell. 2007; 26(5):745-752.
63. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA and Hannon GJ. A microRNA component of the p53 tumour suppressor network. Nature. 2007; 447(7148):1130-1134.
64. Valencia-Sanchez MA, Liu J, Hannon GJ and Parker R. Control of translation and mRNA degradation by miRNAs and siRNAs. Genes Dev. 2006; 20(5):515-524.

65. Peters L and Meister G. Argonaute proteins: mediators of RNA silencing. Mol Cell. 2007; 26(5):611-623.
66. Rokavec M, Oner MG, Li H, Jackstadt R, Jiang L, Lodygin D, Kaller M, Horst D, Ziegler PK, Schwitalla S, Slotta-Huspenina J, Bader FG, Greten FR and Hermeking H. IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. J Clin Invest. 2014; 124(4):1853-1867.
67. Donehower LA HM, Vogel H, McArthur MJ, Montgomery CA Jr, Park SH, Thompson T, Ford RJ, Bradley A. Effects of Genetic Background on Tumorigenesis in p53 deficient mice. Mol CArcinog. 1995; 14(1):16-22.
68. Donehower LA HM, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature. 1992; 356(6366):7.
69. Zhou XZ, Wong S, Walter J, Jacks T and Eisen HN. Increased generation of CD8(+) T cell clones in p53 mutant mice. Journal of Immunology. 1999; 162(7):3957-3960.
70. Gimeno R, Weijer K, Voordouw A, Uittenbogaart CH, Legrand N, Alves NL, Wijnands E, Blom B and Spits H. Monitoring the effect of gene silencing by RNA interference in human CD34+ cells injected into newborn RAG2-/- gammac-/- mice: functional inactivation of p53 in developing T cells. Blood. 2004; 104(13):3886-3893.
71. Li L, Ng DS, Mah WC, Almeida FF, Rahmat SA, Rao VK, Leow SC, Laudisi F, Peh MT, Goh AM, Lim JS, Wright GD, Mortellaro A, Taneja R, Ginhoux F, Lee CG, et al. A unique role for p53 in the regulation of M2 macrophage polarization. Cell Death Differ. 2015; 22(7):1081-1093.
72. Haskins K, Kappler J and Marrack P. The major histocompatibility complex-restricted antigen receptor on T cells. Annu Rev Immunol. 1984; 2:51-66.
73. Cantrell D. T cell antigen receptor signal transduction pathways. Annu Rev Immunol. 1996; 14:259-274.
74. Palmer E. Negative selection--clearing out the bad apples from the T-cell repertoire. Nat Rev Immunol. 2003; 3(5):383-391.
75. Mosmann TR and Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol. 1989; 7:145-173.
76. Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol. 1995; 13:251-276.
77. Wei J, Duramad O, Perng OA, Reiner SL, Liu YJ and Qin FX. Antagonistic nature of T helper 1/2 developmental programs in opposing peripheral induction of Foxp3+ regulatory T cells. Proc Natl Acad Sci U S A. 2007; 104(46):18169-18174.
78. Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD and Paul WE. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. J Exp Med. 1990; 172(3):921-929.
79. Rincon M, Anguita J, Nakamura T, Fikrig E and Flavell RA. Interleukin (IL)-6 directs the differentiation of IL-4-producing CD4+ T cells. J Exp Med. 1997; 185(3):461-469.
80. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, Mitsdoerffer M, Strom TB, Elyaman W, Ho IC, Khoury S, Oukka M and Kuchroo VK. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. Nat Immunol. 2008; 9(12):1347-1355.

81. Jones CP, Gregory LG, Causton B, Campbell GA and Lloyd CM. Activin A and TGF-beta promote T(H)9 cell-mediated pulmonary allergic pathology. J Allergy Clin Immunol. 2012; 129(4):1000-1010 e1003.
82. Elyaman W, Bassil R, Bradshaw EM, Orent W, Lahoud Y, Zhu B, Radtke F, Yagita H and Khoury SJ. Notch receptors and Smad3 signaling cooperate in the induction of interleukin-9-producing T cells. Immunity. 2012; 36(4):623-634.
83. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL and Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006; 441(7090):235-238.
84. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM and Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005; 6(11):1123-1132.
85. Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, Cianfarani F, Odorisio T, Traidl-Hoffmann C, Behrendt H, Durham SR, Schmidt-Weber CB and Cavani A. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. J Clin Invest. 2009; 119(12):3573-3585.
86. Crotty S. Follicular helper CD4 T cells (TFH). Annu Rev Immunol. 2011; 29:621-663.
87. Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P and Yamaguchi T. Regulatory T cells: how do they suppress immune responses? Int Immunol. 2009; 21(10):1105-1111.
88. Sakaguchi S, Miyara M, Costantino CM and Hafler DA. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol. 2010; 10(7):490-500.
89. Biron CA and Brossay L. NK cells and NKT cells in innate defense against viral infections. Curr Opin Immunol. 2001; 13(4):458-464.
90. Bevan MJ. Helping the CD8(+) T-cell response. Nat Rev Immunol. 2004; 4(8):595-602.
91. Krammer PH, Arnold R and Lavrik IN. Life and death in peripheral T cells. Nat Rev Immunol. 2007; 7(7):532-542.
92. Benczik M and Gaffen SL. The interleukin (IL)-2 family cytokines: survival and proliferation signaling pathways in T lymphocytes. Immunol Invest. 2004; 33(2):109-142.
93. Verbist KC, Wang R and Green DR. T cell metabolism and the immune response. Semin Immunol. 2012; 24(6):399-404.
94. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA and Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature. 1992; 356(6367):314-317.
95. Nagata S. Mutations in the Fas antigen gene in lpr mice. Semin Immunol. 1994; 6(1):3-8.
96. Peter ME and Krammer PH. The CD95(APO-1/Fas) DISC and beyond. Cell Death Differ. 2003; 10(1):26-35.
97. Li H, Zhu H, Xu CJ and Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. 1998; 94(4):491-501.
98. Cain K, Bratton SB and Cohen GM. The Apaf-1 apoptosome: a large caspase-activating complex. Biochimie. 2002; 84(2-3):203-214.
99. Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, Rimoldi D, French LE and Tschopp J. Inhibition of death receptor signals by cellular FLIP. Nature. 1997; 388(6638):190-195.
100. Budd RC, Yeh WC and Tschopp J. cFLIP regulation of lymphocyte activation and development. Nat Rev Immunol. 2006; 6(3):196-204.

101. Djerbi M, Darreh-Shori T, Zhivotovsky B and Grandien A. Characterization of the human FLICE-inhibitory protein locus and comparison of the anti-apoptotic activity of four different flip isoforms. Scand J Immunol. 2001; 54(1-2):180-189.
102. Kirchhoff S, Muller WW, Krueger A, Schmitz I and Krammer PH. TCR-mediated up-regulation of c-FLIPshort correlates with resistance toward CD95-mediated apoptosis by blocking death-inducing signaling complex activity. J Immunol. 2000; 165(11):6293-6300.
103. Hildeman DA, Zhu Y, Mitchell TC, Kappler J and Marrack P. Molecular mechanisms of activated T cell death in vivo. Curr Opin Immunol. 2002; 14(3):354-359.
104. Hildeman DA, Zhu Y, Mitchell TC, Bouillet P, Strasser A, Kappler J and Marrack P. Activated T cell death in vivo mediated by proapoptotic bcl-2 family member bim. Immunity. 2002; 16(6):759-767.
105. Erlacher M, Michalak EM, Kelly PN, Labi V, Niederegger H, Coultas L, Adams JM, Strasser A and Villunger A. BH3-only proteins Puma and Bim are rate-limiting for gamma-radiation- and glucocorticoid-induced apoptosis of lymphoid cells in vivo. Blood. 2005; 106(13):4131-4138.
106. Van Parijs L, Refaeli Y, Lord JD, Nelson BH, Abbas AK and Baltimore D. Uncoupling IL-2 signals that regulate T cell proliferation, survival, and Fas-mediated activation-induced cell death. Immunity. 1999; 11(3):281-288.
107. Refaeli Y, Van Parijs L, London CA, Tschopp J and Abbas AK. Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis. Immunity. 1998; 8(5):615-623.
108. Ku CC, Murakami M, Sakamoto A, Kappler J and Marrack P. Control of homeostasis of CD8+ memory T cells by opposing cytokines. Science. 2000; 288(5466):675-678.
109. Brenner D, Krammer PH and Arnold R. Concepts of activated T cell death. Crit Rev Oncol Hematol. 2008; 66(1):52-64.
110. Rathmell JC, Farkash EA, Gao W and Thompson CB. IL-7 enhances the survival and maintains the size of naive T cells. J Immunol. 2001; 167(12):6869-6876.
111. Burkitt D. A sarcoma involving the jaws in African children. Br J Surg. 1958; 46(197):218-223.
112. Henle W, Diehl V, Kohn G, Zur Hausen H and Henle G. Herpes-type virus and chromosome marker in normal leukocytes after growth with irradiated Burkitt cells. Science. 1967; 157(3792):1064-1065.
113. Pope JH, Horne MK and Scott W. Transformation of foetal human keukocytes in vitro by filtrates of a human leukaemic cell line containing herpes-like virus. Int J Cancer. 1968; 3(6):857-866.
114. Nemerow GR, Mold C, Schwend VK, Tollefson V and Cooper NR. Identification of gp350 as the viral glycoprotein mediating attachment of Epstein-Barr virus (EBV) to the EBV/C3d receptor of B cells: sequence homology of gp350 and C3 complement fragment C3d. J Virol. 1987; 61(5):1416-1420.
115. Mullen MM, Haan KM, Longnecker R and Jardetzky TS. Structure of the Epstein-Barr virus gp42 protein bound to the MHC class II receptor HLA-DR1. Mol Cell. 2002; 9(2):375-385.
116. Xiao J, Palefsky JM, Herrera R and Tugizov SM. Characterization of the Epstein-Barr virus glycoprotein BMRF-2. Virology. 2007; 359(2):382-396.

117. Klein E, Kis LL and Klein G. Epstein-Barr virus infection in humans: from harmless to life endangering virus-lymphocyte interactions. Oncogene. 2007; 26(9):1297-1305.
118. Altmann M and Hammerschmidt W. Epstein-Barr virus provides a new paradigm: a requirement for the immediate inhibition of apoptosis. PLoS Biol. 2005; 3(12):e404.
119. Sugden B and Warren N. A promoter of Epstein-Barr virus that can function during latent infection can be transactivated by EBNA-1, a viral protein required for viral DNA replication during latent infection. J Virol. 1989; 63(6):2644-2649.
120. Komano J, Sugiura M and Takada K. Epstein-Barr virus contributes to the malignant phenotype and to apoptosis resistance in Burkitt's lymphoma cell line Akata. J Virol. 1998; 72(11):9150-9156.
121. Zimber-Strobl U and Strobl LJ. EBNA2 and Notch signalling in Epstein-Barr virus mediated immortalization of B lymphocytes. Semin Cancer Biol. 2001; 11(6):423-434.
122. Wang D, Liebowitz D and Kieff E. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. Cell. 1985; 43(3 Pt 2):831-840.
123. Henkel T, Ling PD, Hayward SD and Peterson MG. Mediation of Epstein-Barr virus EBNA2 transactivation by recombination signal-binding protein J kappa. Science. 1994; 265(5168):92-95.
124. Sakai T, Taniguchi Y, Tamura K, Minoguchi S, Fukuhara T, Strobl LJ, Zimber-Strobl U, Bornkamm GW and Honjo T. Functional replacement of the intracellular region of the Notch1 receptor by Epstein-Barr virus nuclear antigen 2. J Virol. 1998; 72(7):6034-6039.
125. Parker GA, Crook T, Bain M, Sara EA, Farrell PJ and Allday MJ. Epstein-Barr virus nuclear antigen (EBNA)3C is an immortalizing oncoprotein with similar properties to adenovirus E1A and papillomavirus E7. Oncogene. 1996; 13(12):2541-2549.
126. Kashuba E, Yurchenko M, Yenamandra SP, Snopok B, Szekely L, Bercovich B, Ciechanover A and Klein G. Epstein-Barr virus-encoded EBNA-5 forms trimolecular protein complexes with MDM2 and p53 and inhibits the transactivating function of p53. Int J Cancer. 2011; 128(4):817-825.
127. Uchida J, Yasui T, Takaoka-Shichijo Y, Muraoka M, Kulwichit W, Raab-Traub N and Kikutani H. Mimicry of CD40 signals by Epstein-Barr virus LMP1 in B lymphocyte responses. Science. 1999; 286(5438):300-303.
128. Huen DS, Henderson SA, Croom-Carter D and Rowe M. The Epstein-Barr virus latent membrane protein-1 (LMP1) mediates activation of NF-kappa B and cell surface phenotype via two effector regions in its carboxy-terminal cytoplasmic domain. Oncogene. 1995; 10(3):549-560.
129. Snow AL, Lambert SL, Natkunam Y, Esquivel CO, Krams SM and Martinez OM. EBV can protect latently infected B cell lymphomas from death receptor-induced apoptosis. J Immunol. 2006; 177(5):3283-3293.
130. Wakisaka N, Kondo S, Yoshizaki T, Murono S, Furukawa M and Pagano JS. Epstein-Barr virus latent membrane protein 1 induces synthesis of hypoxia-inducible factor 1 alpha. Mol Cell Biol. 2004; 24(12):5223-5234.
131. Li HP and Chang YS. Epstein-Barr virus latent membrane protein 1: structure and functions. J Biomed Sci. 2003; 10(5):490-504.
132. Tsai CN, Tsai CL, Tse KP, Chang HY and Chang YS. The Epstein-Barr virus oncogene product, latent membrane protein 1, induces the downregulation of E-cadherin gene

expression via activation of DNA methyltransferases. Proc Natl Acad Sci U S A. 2002; 99(15):10084-10089.
133. Panagopoulos D, Victoratos P, Alexiou M, Kollias G and Mosialos G. Comparative analysis of signal transduction by CD40 and the Epstein-Barr virus oncoprotein LMP1 in vivo. J Virol. 2004; 78(23):13253-13261.
134. Wang S, Rowe M and Lundgren E. Expression of the Epstein Barr virus transforming protein LMP1 causes a rapid and transient stimulation of the Bcl-2 homologue Mcl-1 levels in B-cell lines. Cancer Res. 1996; 56(20):4610-4613.
135. Gires O, Zimber-Strobl U, Gonnella R, Ueffing M, Marschall G, Zeidler R, Pich D and Hammerschmidt W. Latent membrane protein 1 of Epstein-Barr virus mimics a constitutively active receptor molecule. EMBO J. 1997; 16(20):6131-6140.
136. Fahraeus R, Fu HL, Ernberg I, Finke J, Rowe M, Klein G, Falk K, Nilsson E, Yadav M, Busson P and et al. Expression of Epstein-Barr virus-encoded proteins in nasopharyngeal carcinoma. Int J Cancer. 1988; 42(3):329-338.
137. Young LS, Dawson CW, Clark D, Rupani H, Busson P, Tursz T, Johnson A and Rickinson AB. Epstein-Barr virus gene expression in nasopharyngeal carcinoma. J Gen Virol. 1988; 69 (Pt 5):1051-1065.
138. Nonkwelo C, Skinner J, Bell A, Rickinson A and Sample J. Transcription start sites downstream of the Epstein-Barr virus (EBV) Fp promoter in early-passage Burkitt lymphoma cells define a fourth promoter for expression of the EBV EBNA-1 protein. J Virol. 1996; 70(1):623-627.
139. Schaefer BC, Strominger JL and Speck SH. Redefining the Epstein-Barr virus-encoded nuclear antigen EBNA-1 gene promoter and transcription initiation site in group I Burkitt lymphoma cell lines. Proc Natl Acad Sci U S A. 1995; 92(23):10565-10569.
140. Tsai CN, Liu ST and Chang YS. Identification of a novel promoter located within the Bam HI Q region of the Epstein-Barr virus genome for the EBNA 1 gene. DNA Cell Biol. 1995; 14(9):767-776.
141. Takada K, Yamamoto K and Osato T. Analysis of the transformation of human lymphocytes by Epstein-Barr virus. II. Abortive response of leukemic cells to the transforming virus. Intervirology. 1980; 13(4):223-231.
142. Rickinson AB, Finerty S and Epstein MA. Interaction of Epstein-Barr virus with leukaemic B cells in vitro. I. Abortive infection and rare cell line establishment from chronic lymphocytic leukaemic cells. Clin Exp Immunol. 1982; 50(2):347-354.
143. Davenport MG and Pagano JS. Expression of EBNA-1 mRNA is regulated by cell cycle during Epstein-Barr virus type I latency. J Virol. 1999; 73(4):3154-3161.
144. Lee MA, Diamond ME and Yates JL. Genetic evidence that EBNA-1 is needed for efficient, stable latent infection by Epstein-Barr virus. J Virol. 1999; 73(4):2974-2982.
145. Lindner SE and Sugden B. The plasmid replicon of Epstein-Barr virus: mechanistic insights into efficient, licensed, extrachromosomal replication in human cells. Plasmid. 2007; 58(1):1-12.
146. Klein G and Klein E. Surveillance against tumors--is it mainly immunological? Immunol Lett. 2005; 100(1):29-33.
147. Callan MF, Steven N, Krausa P, Wilson JD, Moss PA, Gillespie GM, Bell JI, Rickinson AB and McMichael AJ. Large clonal expansions of CD8+ T cells in acute infectious mononucleosis. Nat Med. 1996; 2(8):906-911.

148. Murray RJ, Kurilla MG, Brooks JM, Thomas WA, Rowe M, Kieff E and Rickinson AB. Identification of target antigens for the human cytotoxic T cell response to Epstein-Barr virus (EBV): implications for the immune control of EBV-positive malignancies. J Exp Med. 1992; 176(1):157-168.
149. Khanna R, Burrows SR, Steigerwald-Mullen PM, Thomson SA, Kurilla MG and Moss DJ. Isolation of cytotoxic T lymphocytes from healthy seropositive individuals specific for peptide epitopes from Epstein-Barr virus nuclear antigen 1: implications for viral persistence and tumor surveillance. Virology. 1995; 214(2):633-637.
150. Levitskaya J, Coram M, Levitsky V, Imreh S, Steigerwald-Mullen PM, Klein G, Kurilla MG and Masucci MG. Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. Nature. 1995; 375(6533):685-688.
151. Khanna R, Burrows SR, Steigerwald-Mullen PM, Moss DJ, Kurilla MG and Cooper L. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA1) through the class II pathway restores immune recognition by EBNA1-specific cytotoxic T lymphocytes: evidence for HLA-DM-independent processing. Int Immunol. 1997; 9(10):1537-1543.
152. Leen A, Meij P, Redchenko I, Middeldorp J, Bloemena E, Rickinson A and Blake N. Differential immunogenicity of Epstein-Barr virus latent-cycle proteins for human CD4(+) T-helper 1 responses. J Virol. 2001; 75(18):8649-8659.
153. Paludan C, Bickham K, Nikiforow S, Tsang ML, Goodman K, Hanekom WA, Fonteneau JF, Stevanovic S and Munz C. Epstein-Barr nuclear antigen 1-specific CD4(+) Th1 cells kill Burkitt's lymphoma cells. J Immunol. 2002; 169(3):1593-1603.
154. Voo KS, Fu T, Heslop HE, Brenner MK, Rooney CM and Wang RF. Identification of HLA-DP3-restricted peptides from EBNA1 recognized by CD4(+) T cells. Cancer Res. 2002; 62(24):7195-7199.
155. Maini MK, Gudgeon N, Wedderburn LR, Rickinson AB and Beverley PC. Clonal expansions in acute EBV infection are detectable in the CD8 and not the CD4 subset and persist with a variable CD45 phenotype. J Immunol. 2000; 165(10):5729-5737.
156. Long HM, Haigh TA, Gudgeon NH, Leen AM, Tsang CW, Brooks J, Landais E, Houssaint E, Lee SP, Rickinson AB and Taylor GS. CD4+ T-cell responses to Epstein-Barr virus (EBV) latent-cycle antigens and the recognition of EBV-transformed lymphoblastoid cell lines. J Virol. 2005; 79(8):4896-4907.
157. Precopio ML, Sullivan JL, Willard C, Somasundaran M and Luzuriaga K. Differential kinetics and specificity of EBV-specific CD4+ and CD8+ T cells during primary infection. J Immunol. 2003; 170(5):2590-2598.
158. Munz C, Bickham KL, Subklewe M, Tsang ML, Chahroudi A, Kurilla MG, Zhang D, O'Donnell M and Steinman RM. Human CD4(+) T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1. J Exp Med. 2000; 191(10):1649-1660.
159. Khan G, Miyashita EM, Yang B, Babcock GJ and Thorley-Lawson DA. Is EBV persistence in vivo a model for B cell homeostasis? Immunity. 1996; 5(2):173-179.
160. Hochberg D, Souza T, Catalina M, Sullivan JL, Luzuriaga K and Thorley-Lawson DA. Acute infection with Epstein-Barr virus targets and overwhelms the peripheral memory B-cell compartment with resting, latently infected cells. J Virol. 2004; 78(10):5194-5204.
161. Babcock GJ, Hochberg D and Thorley-Lawson AD. The expression pattern of Epstein-Barr virus latent genes in vivo is dependent upon the differentiation stage of the infected B cell. Immunity. 2000; 13(4):497-506.

162. Thorley-Lawson DA. Epstein-Barr virus: exploiting the immune system. Nat Rev Immunol. 2001; 1(1):75-82.
163. Rajkumar SV. Treatment of multiple myeloma. Nat Rev Clin Oncol. 2011; 8(8):479-491.
164. Nishida K, Tamura A, Nakazawa N, Ueda Y, Abe T, Matsuda F, Kashima K and Taniwaki M. The Ig heavy chain gene is frequently involved in chromosomal translocations in multiple myeloma and plasma cell leukemia as detected by in situ hybridization. Blood. 1997; 90(2):526-534.
165. Malgeri U, Baldini L, Perfetti V, Fabris S, Vignarelli MC, Colombo G, Lotti V, Compasso S, Bogni S, Lombardi L, Maiolo AT and Neri A. Detection of t(4;14)(p16.3;q32) chromosomal translocation in multiple myeloma by reverse transcription-polymerase chain reaction analysis of IGH-MMSET fusion transcripts. Cancer Res. 2000; 60(15):4058-4061.
166. Kuehl WM and Bergsagel PL. Multiple myeloma: evolving genetic events and host interactions. Nat Rev Cancer. 2002; 2(3):175-187.
167. Bergsagel PL and Kuehl WM. Molecular pathogenesis and a consequent classification of multiple myeloma. J Clin Oncol. 2005; 23(26):6333-6338.
168. Ramakrishnan V, Timm M, Haug JL, Kimlinger TK, Wellik LE, Witzig TE, Rajkumar SV, Adjei AA and Kumar S. Sorafenib, a dual Raf kinase/vascular endothelial growth factor receptor inhibitor has significant anti-myeloma activity and synergizes with common anti-myeloma drugs. Oncogene. 2010; 29(8):1190-1202.
169. Hardin J, MacLeod S, Grigorieva I, Chang R, Barlogie B, Xiao H and Epstein J. Interleukin-6 prevents dexamethasone-induced myeloma cell death. Blood. 1994; 84(9):3063-3070.
170. Puthier D, Bataille R and Amiot M. IL-6 up-regulates mcl-1 in human myeloma cells through JAK / STAT rather than ras / MAP kinase pathway. Eur J Immunol. 1999; 29(12):3945-3950.
171. Adams J. The proteasome: a suitable antineoplastic target. Nat Rev Cancer. 2004; 4(5):349-360.
172. Quach H, Ritchie D, Stewart AK, Neeson P, Harrison S, Smyth MJ and Prince HM. Mechanism of action of immunomodulatory drugs (IMiDS) in multiple myeloma. Leukemia. 2010; 24(1):22-32.
173. Chauhan D, Hideshima T, Mitsiades C, Richardson P and Anderson KC. Proteasome inhibitor therapy in multiple myeloma. Mol Cancer Ther. 2005; 4(4):686-692.
174. Chauhan D, Hideshima T and Anderson KC. Proteasome inhibition in multiple myeloma: therapeutic implication. Annu Rev Pharmacol Toxicol. 2005; 45:465-476.
175. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973; 243(5405):290-293.
176. Nowell PC. Discovery of the Philadelphia chromosome: a personal perspective. J Clin Invest. 2007; 117(8):2033-2035.
177. Melo JV and Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. Nat Rev Cancer. 2007; 7(6):441-453.
178. Cohen GB, Ren R and Baltimore D. Modular binding domains in signal transduction proteins. Cell. 1995; 80(2):237-248.

179. McWhirter JR and Wang JY. An actin-binding function contributes to transformation by the Bcr-Abl oncoprotein of Philadelphia chromosome-positive human leukemias. EMBO J. 1993; 12(4):1533-1546.
180. Heltemes-Harris LM, Willette MJ, Vang KB and Farrar MA. The role of STAT5 in the development, function, and transformation of B and T lymphocytes. Ann N Y Acad Sci. 2011; 1217:18-31.
181. Puil L, Liu J, Gish G, Mbamalu G, Bowtell D, Pelicci PG, Arlinghaus R and Pawson T. Bcr-Abl oncoproteins bind directly to activators of the Ras signalling pathway. EMBO J. 1994; 13(4):764-773.
182. Talpaz M, McCredie KB, Mavligit GM and Gutterman JU. Leukocyte interferon-induced myeloid cytoreduction in chronic myelogenous leukemia. Blood. 1983; 62(3):689-692.
183. Talpaz M, Kantarjian HM, McCredie KB, Keating MJ, Trujillo J and Gutterman J. Clinical investigation of human alpha interferon in chronic myelogenous leukemia. Blood. 1987; 69(5):1280-1288.
184. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J and Lydon NB. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996; 2(5):561-566.
185. Aswald JM, Lipton JH, Aswald S and Messner HA. Increased IFN-gamma synthesis by T cells from patients on imatinib therapy for chronic myeloid leukemia. Cytokines Cell Mol Ther. 2002; 7(4):143-149.
186. Chomel JC and Turhan AG. Chronic myeloid leukemia stem cells in the era of targeted therapies: resistance, persistence and long-term dormancy. Oncotarget. 2011; 2(9):713-727.
187. Isaacs A and Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci. 1957; 147(927):258-267.
188. Kerr IM and Stark GR. The control of interferon-inducible gene expression. FEBS Lett. 1991; 285(2):194-198.
189. Kovarik P, Stoiber D, Novy M and Decker T. Stat1 combines signals derived from IFN-gamma and LPS receptors during macrophage activation. EMBO J. 1998; 17(13):3660-3668.
190. Sekimoto T, Imamoto N, Nakajima K, Hirano T and Yoneda Y. Extracellular signal-dependent nuclear import of Stat1 is mediated by nuclear pore-targeting complex formation with NPI-1, but not Rch1. EMBO J. 1997; 16(23):7067-7077.
191. Ohmori Y and Hamilton TA. IFN-gamma selectively inhibits lipopolysaccharide-inducible JE/monocyte chemoattractant protein-1 and KC/GRO/melanoma growth-stimulating activity gene expression in mouse peritoneal macrophages. J Immunol. 1994; 153(5):2204-2212.
192. Bach EA, Aguet M and Schreiber RD. The IFN gamma receptor: a paradigm for cytokine receptor signaling. Annu Rev Immunol. 1997; 15:563-591.
193. Jonasch E and Haluska FG. Interferon in oncological practice: review of interferon biology, clinical applications, and toxicities. Oncologist. 2001; 6(1):34-55.
194. Young HA. Regulation of interferon-gamma gene expression. J Interferon Cytokine Res. 1996; 16(8):563-568.

195. Carnaud C, Lee D, Donnars O, Park SH, Beavis A, Koezuka Y and Bendelac A. Cutting edge: Cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. J Immunol. 1999; 163(9):4647-4650.
196. Flaishon L, Hershkoviz R, Lantner F, Lider O, Alon R, Levo Y, Flavell RA and Shachar I. Autocrine secretion of interferon gamma negatively regulates homing of immature B cells. J Exp Med. 2000; 192(9):1381-1388.
197. Kaplan DH, Greenlund AC, Tanner JW, Shaw AS and Schreiber RD. Identification of an interferon-gamma receptor alpha chain sequence required for JAK-1 binding. J Biol Chem. 1996; 271(1):9-12.
198. Bach EA, Tanner JW, Marsters S, Ashkenazi A, Aguet M, Shaw AS and Schreiber RD. Ligand-induced assembly and activation of the gamma interferon receptor in intact cells. Mol Cell Biol. 1996; 16(6):3214-3221.
199. Greenlund AC, Morales MO, Viviano BL, Yan H, Krolewski J and Schreiber RD. Stat recruitment by tyrosine-phosphorylated cytokine receptors: an ordered reversible affinity-driven process. Immunity. 1995; 2(6):677-687.
200. Briscoe J, Rogers NC, Witthuhn BA, Watling D, Harpur AG, Wilks AF, Stark GR, Ihle JN and Kerr IM. Kinase-negative mutants of JAK1 can sustain interferon-gamma-inducible gene expression but not an antiviral state. EMBO J. 1996; 15(4):799-809.
201. Darnell JE, Jr., Kerr IM and Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science. 1994; 264(5164):1415-1421.
202. Ramana CV, Chatterjee-Kishore M, Nguyen H and Stark GR. Complex roles of Stat1 in regulating gene expression. Oncogene. 2000; 19(21):2619-2627.
203. McBride KM and Reich NC. The ins and outs of STAT1 nuclear transport. Sci STKE. 2003; 2003(195):RE13.
204. Vinkemeier U. Getting the message across, STAT! Design principles of a molecular signaling circuit. J Cell Biol. 2004; 167(2):197-201.
205. Kisseleva T, Bhattacharya S, Braunstein J and Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene. 2002; 285(1-2):1-24.
206. Mustelin T, Vang T and Bottini N. Protein tyrosine phosphatases and the immune response. Nat Rev Immunol. 2005; 5(1):43-57.
207. Nakahira M, Tanaka T, Robson BE, Mizgerd JP and Grusby MJ. Regulation of signal transducer and activator of transcription signaling by the tyrosine phosphatase PTP-BL. Immunity. 2007; 26(2):163-176.
208. McBride KM, McDonald C and Reich NC. Nuclear export signal located within theDNA-binding domain of the STAT1transcription factor. EMBO J. 2000; 19(22):6196-6206.
209. Yoshimura A, Naka T and Kubo M. SOCS proteins, cytokine signalling and immune regulation. Nat Rev Immunol. 2007; 7(6):454-465.
210. Iwamoto T, Senga T, Naito Y, Matsuda S, Miyake Y, Yoshimura A and Hamaguchi M. The JAK-inhibitor, JAB/SOCS-1 selectively inhibits cytokine-induced, but not v-Src induced JAK-STAT activation. Oncogene. 2000; 19(41):4795-4801.
211. Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J and Paschke R. Insulin resistance-inducing cytokines differentially regulate SOCS mRNA expression via growth factor- and Jak/Stat-signaling pathways in 3T3-L1 adipocytes. J Endocrinol. 2004; 181(1):129-138.

212. Krebs DL and Hilton DJ. SOCS proteins: negative regulators of cytokine signaling. Stem Cells. 2001; 19(5):378-387.
213. Decker T and Kovarik P. Serine phosphorylation of STATs. Oncogene. 2000; 19(21):2628-2637.
214. Wang D, Moriggl R, Stravopodis D, Carpino N, Marine JC, Teglund S, Feng J and Ihle JN. A small amphipathic alpha-helical region is required for transcriptional activities and proteasome-dependent turnover of the tyrosine-phosphorylated Stat5. EMBO J. 2000; 19(3):392-399.
215. Tenoever BR, Ng SL, Chua MA, McWhirter SM, Garcia-Sastre A and Maniatis T. Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. Science. 2007; 315(5816):1274-1278.
216. Varinou L, Ramsauer K, Karaghiosoff M, Kolbe T, Pfeffer K, Muller M and Decker T. Phosphorylation of the Stat1 transactivation domain is required for full-fledged IFN-gamma-dependent innate immunity. Immunity. 2003; 19(6):793-802.
217. Morinobu A, Gadina M, Strober W, Visconti R, Fornace A, Montagna C, Feldman GM, Nishikomori R and O'Shea JJ. STAT4 serine phosphorylation is critical for IL-12-induced IFN-gamma production but not for cell proliferation. Proc Natl Acad Sci U S A. 2002; 99(19):12281-12286.
218. Nadiminty N, Lou W, Lee SO, Lin X, Trump DL and Gao AC. Stat3 activation of NF-{kappa}B p100 processing involves CBP/p300-mediated acetylation. Proc Natl Acad Sci U S A. 2006; 103(19):7264-7269.
219. Yuan ZL, Guan YJ, Chatterjee D and Chin YE. Stat3 dimerization regulated by reversible acetylation of a single lysine residue. Science. 2005; 307(5707):269-273.
220. Wang R, Cherukuri P and Luo J. Activation of Stat3 sequence-specific DNA binding and transcription by p300/CREB-binding protein-mediated acetylation. J Biol Chem. 2005; 280(12 :11528-11534.
221. Gewinner C, Hart G, Zachara N, Cole R, Beisenherz-Huss C and Groner B. The coactivator of transcription CREB-binding protein interacts preferentially with the glycosylated form of Stat5. J Biol Chem. 2004; 279(5):3563-3572.
222. Bardwell VJ and Treisman R. The POZ domain: a conserved protein-protein interaction motif. Genes Dev. 1994; 8(14):1664-1677.
223. Niu H, Ye BH and Dalla-Favera R. Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcription factor. Genes Dev. 1998; 12(13):1953-1961.
224. Wong CW and Privalsky ML. Components of the SMRT corepressor complex exhibit distinctive interactions with the POZ domain oncoproteins PLZF, PLZF-RARalpha, and BCL-6. J Biol Chem. 1998; 273(42):27695-27702.
225. Lemercier C, Brocard MP, Puvion-Dutilleul F, Kao HY, Albagli O and Khochbin S. Class II histone deacetylases are directly recruited by BCL6 transcriptional repressor. J Biol Chem. 2002; 277(24):22045-22052.
226. Basso K, Saito M, Sumazin P, Margolin AA, Wang K, Lim WK, Kitagawa Y, Schneider C, Alvarez MJ, Califano A and Dalla-Favera R. Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells. Blood. 2010; 115(5):975-984.
227. Ci W, Polo JM, Cerchietti L, Shaknovich R, Wang L, Yang SN, Ye K, Farinha P, Horsman DE, Gascoyne RD, Elemento O and Melnick A. The BCL6 transcriptional

program features repression of multiple oncogenes in primary B cells and is deregulated in DLBCL. Blood. 2009; 113(22):5536-5548.
228. Bunting KL and Melnick AM. New effector functions and regulatory mechanisms of BCL6 in normal and malignant lymphocytes. Curr Opin Immunol. 2013; 25(3):339-346.
229. Basso K and Dalla-Favera R. BCL6: master regulator of the germinal center reaction and key oncogene in B cell lymphomagenesis. Adv Immunol. 2010; 105:193-210.
230. Khanna KK and Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet. 2001; 27(3):247-254.
231. Phan RT and Dalla-Favera R. The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells. Nature. 2004; 432(7017):635-639.
232. Pasqualucci L, Migliazza A, Basso K, Houldsworth J, Chaganti RS and Dalla-Favera R. Mutations of the BCL6 proto-oncogene disrupt its negative autoregulation in diffuse large B-cell lymphoma. Blood. 2003; 101(8):2914-2923.
233. Walker SR, Nelson EA and Frank DA. STAT5 represses BCL6 expression by binding to a regulatory region frequently mutated in lymphomas. Oncogene. 2007; 26(2):224-233.
234. Walker SR, Nelson EA, Yeh JE, Pinello L, Yuan GC and Frank DA. STAT5 outcompetes STAT3 to regulate the expression of the oncogenic transcriptional modulator BCL6. Mol Cell Biol. 2013; 33(15):2879-2890.
235. Bereshchenko OR, Gu W and Dalla-Favera R. Acetylation inactivates the transcriptional repressor BCL6. Nat Genet. 2002; 32(4):606-613.
236. Saito M, Gao J, Basso K, Kitagawa Y, Smith PM, Bhagat G, Pernis A, Pasqualucci L and Dalla-Favera R. A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma. Cancer Cell. 2007; 12(3):280-292.
237. Ye BH, Rao PH, Chaganti RS and Dalla-Favera R. Cloning of bcl-6, the locus involved in chromosome translocations affecting band 3q27 in B-cell lymphoma. Cancer Res. 1993; 53(12):2732-2735.
238. Chen W, Iida S, Louie DC, Dalla-Favera R and Chaganti RS. Heterologous promoters fused to BCL6 by chromosomal translocations affecting band 3q27 cause its deregulated expression during B-cell differentiation. Blood. 1998; 91(2):603-607.
239. Pasqualucci L, Bhagat G, Jankovic M, Compagno M, Smith P, Muramatsu M, Honjo T, Morse HC, 3rd, Nussenzweig MC and Dalla-Favera R. AID is required for germinal center-derived lymphomagenesis. Nat Genet. 2008; 40(1):108-112.
240. Nagy N, Takahara M, Nishikawa J, Bourdon JC, Kis LL, Klein G and Klein E. Wild-type p53 activates SAP expression in lymphoid cells. Oncogene. 2004; 23(53):8563-8570.
241. Nagy N, Matskova L, Kis LL, Hellman U, Klein G and Klein E. The proapoptotic function of SAP provides a clue to the clinical picture of X-linked lymphoproliferative disease. Proc Natl Acad Sci U S A. 2009; 106(29):11966-11971.
242. Snow AL, Marsh RA, Krummey SM, Roehrs P, Young LR, Zhang K, van Hoff J, Dhar D, Nichols KE, Filipovich AH, Su HC, Bleesing JJ and Lenardo MJ. Restimulation-induced apoptosis of T cells is impaired in patients with X-linked lymphoproliferative disease caused by SAP deficiency. J Clin Invest. 2009; 119(10):2976-2989.
243.Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N and Liu EA. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science. 2004; 303(5659):844-848.

244. Mustata G, Follis AV, Hammoudeh DI, Metallo SJ, Wang H, Prochownik EV, Lazo JS and Bahar I. Discovery of novel Myc-Max heterodimer disruptors with a three-dimensional pharmacophore model. J Med Chem. 2009; 52(5):1247-1250.
245. Geling A, Steiner H, Willem M, Bally-Cuif L and Haass C. A gamma-secretase inhibitor blocks Notch signaling in vivo and causes a severe neurogenic phenotype in zebrafish. EMBO Rep. 2002; 3(7):688-694.
246. Kis LL, Salamon D, Persson EK, Nagy N, Scheeren FA, Spits H, Klein G and Klein E. IL-21 imposes a type II EBV gene expression on type III and type I B cells by the repression of C- and activation of LMP-1-promoter. Proc Natl Acad Sci U S A. 2010; 107(2):872-877.
247. Pokrovskaja K, Ehlin-Henriksson B, Kiss C, Challa A, Gordon J, Gogolak P, Klein G and Szekely L. CD40 ligation downregulates EBNA-2 and LMP-1 expression in EBV-transformed lymphoblastoid cell lines. Int J Cancer. 2002; 99(5):705-712.
248. Zhou G and Ono SJ. Induction of BCL-6 gene expression by interferon-gamma and identification of an IRE in exon I. Exp Mol Pathol. 2005; 78(1):25-35.
249. Lee SK, Silva DG, Martin JL, Pratama A, Hu X, Chang PP, Walters G and Vinuesa CG. Interferon-gamma excess leads to pathogenic accumulation of follicular helper T cells and germinal centers. Immunity. 2012; 37(5):880-892.
250. Huang BB, Bonish BK, Chaturvedi V, Qin JZ and Nickoloff BJ. Keratinocyte CDw60 expression is modulated by both a Th-1 type cytokine IFN-gamma and Th-2 cytokines IL-4 and IL-13: relevance to psoriasis. J Invest Dermatol. 2001; 116(2):305-312.
251. John S, Vinkemeier U, Soldaini E, Darnell JE, Jr. and Leonard WJ. The significance of tetramerization in promoter recruitment by Stat5. Mol Cell Biol. 1999; 19(3):1910-1918.
252. Mandal M, Powers SE, Maienschein-Cline M, Bartom ET, Hamel KM, Kee BL, Dinner AR and Clark MR. Epigenetic repression of the Igk locus by STAT5-mediated recruitment of the histone methyltransferase Ezh2. Nat Immunol. 2011; 12(12):1212-1220.
253. Bollard, C.M., C.M. Rooney, and H.E. Heslop, T-cell therapy in the treatment of post-transplant lymphoproliferative disease. Nat Rev Clin Oncol, 2012. 9(9): p. 510-9
254. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et.al., Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. Proc Natl Acad Sci U S A. 1998 Nov 10;95(23):13765-70.
255. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, Cahn AP, et.al., Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. Nat Genet. 1998 Oct;20(2):129-35.
256. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, van Schaik S, Notarangelo L, Geha R, Roncarolo MG, Oettgen H, De Vries JE, Aversa G, Terhorst C. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. Nature. 1998 Oct 1;395(6701):462-9.
257. Purtilo DT, Grierson HL, Davis JR, Okano M. The X-linked lymphoproliferative disease: from autopsy toward cloning the gene 1975-1990. Pediatr Pathol. 1991 Sep-Oct;11(5):685-710.
258. Maia DM, Garwacki CP. X-linked lymphoproliferative disease: pathology and diagnosis. Pediatr Dev Pathol. 1999 Jan-Feb;2(1):72-7.

259. Sharifi R1, Sinclair JC, Gilmour KC, Arkwright PD, Kinnon C, Thrasher AJ, Gaspar HB. SAP mediates specific cytotoxic T-cell functions in X-linked lymphoproliferative disease. Blood. 2004 May 15;103(10):3821-7. Epub 2004 Jan 15.
260. Qi H1, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. Nature. 2008 Oct 9;455(7214):764-9. doi: 10.1038/nature07345.